Project Report No. 518 **August 2013**

Current status of soils and responsiveness of wheat to micronutrient applications

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1. Abstract

Most micronutrient trials in the UK were performed when winter wheat yields were less than half those achieved now. Recent publications suggest that modern high-yielding wheat varieties have lower micronutrient concentrations in grain. It is not clear if this is due to changes in the crop or in the micronutrients available in soils.

Over 30 years, the geometric mean total concentration of copper (Cu) in soil decreased by less than 1 mg/kg and manganese (Mn) by nearly 40 mg/kg, but total zinc (Zn) increased by almost 5 mg/kg. Soils were also extracted with EDTA (ethylene diamine tetraacetic acid) and there was only a decrease of just over 1 mg/kg for Cu and Zn, which is not likely to be biologically significant. Manganese extracted by EDTA decreased by nearly 50 mg/kg, but for Mn this extract is not thought to be useful for indicating crop availability.

Fifteen field experiments were performed on soils likely to be deficient in micronutrients (light, calcareous or soil high in organic matter) all sown with winter wheat variety Solstice. Each experiment was treated at the early stem elongation stage with sprays containing Cu, Mn or Zn at the rates recommended for severe deficiency. Only two statistically significant yield responses were observed. These were +0.27 t/ha in the Zn spray treatment at a site on light loamy sand at Morley (P =0.04), and +1.39 t/ha with Cu sprays on a highly organic soil at Waterbeach (P =0.058). EDTA soil extraction gave correct prediction on responsiveness in yield to Cu in one out of two cases where concentrations were classed as low, but only by adopting a critical value for Cu of 1 mg/kg. Leaf analysis in spring was predictive of the one Cu responsive site, but this appears to be less useful for Mn and Zn.

The percentages of responsive trials, although low, are in fact similar to UK recent results of winter wheat trials, selected as those performed since 2005, for Cu (7%) and Mn (0%) but not for Zn, where the percentage was slightly smaller (7% versus 16%). It seems unlikely that modern high-yielding wheat is more responsive in yield to micronutrient applications than older varieties. This may be because most UK soils contain sufficient concentrations of available micronutrients, although there is specific soil and local variation. Although our experiments were not specifically set up to examine the possibility of “growth dilution” of micronutrient concentrations in grain, there was no evidence that the lowest grain micronutrient concentrations were associated with the highest yields in our experiments.

2. Introduction

Micronutrients or trace elements are those nutrients required in small amounts for essential growth processes in plants and animals. These include iron (Fe), Cu, Mn, Zn, boron (B), molybdenum (Mo) and chlorine (Cl). Some micronutrients that are essential for animals are not required by plants, but animals usually acquire them via consumed plant tissues. Occurrences of deficiency are most frequently related to soil type, soil pH, soil structural conditions and their effect on root growth, and also to crop susceptibility. Of the micronutrients, Cu, Mn and Zn are thought to be the most limiting in wheat (Knight et al, 2012).

Low micronutrient concentrations in soil can affect wheat yields (Roques et al, 2013). However the Fertiliser Manual (RB209, 2010) identified that much of the UK research in this area was undertaken in the 1970's and 1980's (ADAS/MAFF, 1984) and as such may not be truly relevant to modern high yielding wheat varieties. Average yields, and therefore the micronutrient requirements of winter wheat, have increased in the last 40 years by at least two-fold (Figure 1). Many crops are producing above average yields and 10-11 t/ha are possible, even under slightly adverse soil conditions (see Results below). The future trend will likely see "sustainable intensification", which requires increasing yields (Royal Society, 2009). In fact, average yields have stagnated during the last 15-20 years (Figure 1), but we know that yields are still well below the genetic potential. The plateau of yields could be due to many factors (Knight et al, 2012). The question arises as to whether micronutrients could be one of those limiting increasing yields, especially in particular soil and management situations?

Visual symptoms of a deficiency of a specific micronutrient can be confused with those produced by other growth problems. Consequently, visual diagnosis of a micronutrient deficiency should, where possible, be confirmed by plant and/or soil analysis. A problem exists in that there are many different critical values used in both this country and overseas, and also different extraction methods for soil analyses. These are also under-researched and their technical basis is often obscure (Roques et al, 2013). One further issue is that they were often promulgated many years ago, when wheat yields were smaller. It appears that increasing yields following the "green revolution may have led to a "growth dilution" of micronutrients in wheat grain (Fan et al, 2008, Gooding et al, 2012, Garvin et al, 2006). Although it is also possible that decreasing total or available micronutrients in soil is also responsible. The question arises whether existing soil nutrient indices and critical plant tissue analyses remain valid, and whether the boundaries have changed in terms of wheat nutrient requirements? Additionally, do modern high-yielding wheats respond in yields to micronutrient applications and do their concentrations in grain increase?

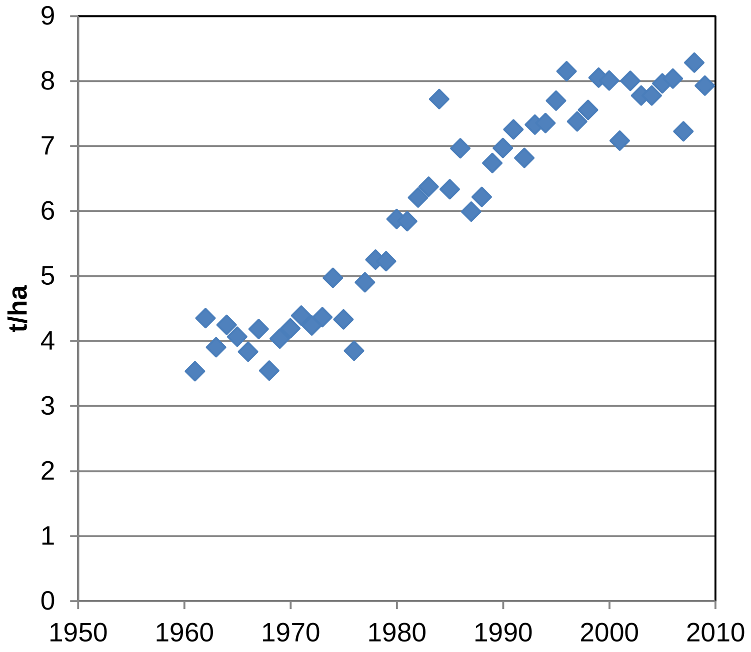


Figure 1. Average yield of winter wheat crops in the UK over the last 50 years (FAO, 2013).

There is currently a lack of reliable recent UK-based literature on the above subjects, and because of this, micronutrient research was prioritised by the HGCA in its mid-term review of R&D strategies for 2007-2010. Research reported here was conducted between 2009 and 2012, and was recently added to by a review of published literature on the responses of cereals and oilseed rape to micronutrients (Roques et al, 2013).

2.1. Objectives

This research is designed to provide recommendations as to whether additions of the micronutrients Cu, Mn and Zn are required, based on the following key lines of research: i) assessing the changes in the total and available micronutrient supply in arable soils over time; and ii) using a programme of field experiments to evaluate whether the old soil index and critical plant tissue values are appropriate to assess the yield-responsiveness of modern high-yielding wheat varieties, and whether new methods of soil analysis can better predict micronutrient supply and yield responsiveness. Outputs from the project were intended to address whether fertiliser additions are needed for yield, and the use of soil and crop testing, which are the key practical measures for improvement. A secondary objective was to determine whether Cu, Mn or Zn spays are effective in biofortifying the concentrations of these micronutrients in wheat grain.

3. Materials and methods

3.1. Soil micronutrient survey

A total of 132 “new” samples of arable soils were taken in England and Wales in 2009/10 and sent to Hill Court Farm Research as part of routine testing. These were not biased towards soils low in micronutrients, as many of the samples were in fact submitted for major nutrient analysis, particularly of mineral N in soil. The aim was to compare soils taken recently with those sampled and analysed around 30 years ago (1978-1982), in the National Soil Inventory, which was published in the Soil Geochemical Atlas of England and Wales (McGrath and Loveland, 1992). The new soils were air dried and sieved to 2 mm before micronutrient analysis at Rothamsted Research.

“Total” major and trace soil elements in soils were measured after fine-grinding in an all-agate planetary ball mill and aqua regia digestion (McGrath and Cunliffe, 1985) and analysed using inductively coupled plasma optical emission spectroscopy or inductively coupled plasma mass spectroscopy (ICP-OES or ICP-MS). Micronutrient concentrations were also determined in 0.05 M EDTA-extracts of soils that had been sieved to 2mm (MAFF, 1986).

3.2. Micronutrient response trials

3.2.1. Field Trials, experimental set up

Field sites were chosen to be on soils that had a risk of micronutrient deficiency: light sandy soils, soils high in organic matter and calcareous soils. These all have either low contents of micronutrients and/or lack of availability of the micronutrients present due to the soil chemical properties. All of the field sites (particularly the organic soils) were locations where micronutrients would be used routinely by farmers. Field experiments were set up with the same design at 5 sites each year over the three harvest years 2010, 2011, 2012 (harvest years), giving a total of 15 site/years. To ensure comparability, all sites were sown with Solstice, which is a nabim group 1 breadmaking winter wheat variety. Details of the sites are given below. N, P, K fertilisers were added on a site basis to avoid limitation and provide sufficient nutrients to support high yields, all plots received adequate S fertiliser (37.5-50 kg/ha SO_3), and standard farm practice for pesticide and herbicide applications and growth regulators. Each plot was at least 2 m x 10 m or 12 m, depending on the site.

The treatments were replicated four-fold and included double control plots (which strengthens the statistical comparisons with control values), Cu, or Mn, or Zn spray treatments, resulting in a total of 20 plots per site. These were laid out in a randomised block design at each site. Treatments used the foliar products supplied by Headland and followed the supplier's instructions on rate and timing of applications. All products were applied in 200 l/ha water using flat fan nozzles. In all cases we used the two applications recommended for cases where severe deficiency is suspected. Rates, timing and total amounts of each element added are given in Table 1.

Table 1. Treatments, rates and timing of micronutrient fertilisers for all wheat experiments, 2010-2012

Treatment	**GS30-31 (dose of product)**	**GS 32 (dose of product)**	**Total metal added (kg/ha)**
Untreated	-	-	-
Untreated	-	-	-
Copper (Headland copper oxychloride as supplied)	0.5 l/ha	0.5 l//ha	0.435
Manganese (Headland Maple DF manganese sulphate as supplied)	1.95 kg/ha	1.95 kg/ha	1.17 (S 0.702)
Zinc (Headland zinc sulphate as supplied)	4.0 l/ha	4.0 l/ha	0.273

3.2.2. Analytical methods

The following types of analysis were made:

- Soil analysis: total-, EDTA-, DPTA-, ammonium nitrate- and DGT-extractable Cu, Mn and Zn
- Co-determinants of micronutrient availability: soil organic matter, available P, pH and texture.
- Plant analysis: micronutrient (Zn, Cu and Mn) concentrations in leaves and grain by acid digestion and ICP measurements.

Soil pH was measured in water with a soil:water ratio of 1:2.5. Available phosphate was determined using Olsen P method (sodium bicarbonate extraction) (Olsen *et al* 1954). "Total" concentrations of major and trace elements in soil were measured as in 2.2.1 above.

Leaf and grain analyses for major and trace elements were made after digestion with nitric/perchloric acids (Zhao *et al* 1994) and determined by ICP-OES.

Total carbon and N in soils was analysed using LECO TruMac macro combustion instrument; organic carbon was calculated after subtracting any inorganic carbon that was measured by manonometery. In addition to EDTA-extractable metals in soil, we also used other methods that may be predictive of deficiencies of micronutrients in soils. These were: DTPA extracts (Lindsey and Norvell, 1978); 1M ammonium nitrate extracts (Prüess 1998) and Diffusive Gradients in Thin film devices (DGT) (Zhang et al, 2001). The DTPA and ammonium nitrate extracts were measured at Rothamsted Research using ICP-OES or MS, depending on the concentrations and DGT devices were eluted and analysed by ICP-MS at Lancaster University.

3.3. Statistical analysis

Both soil and plant results are reported on an air-dry basis, except grain yields which are reported on an 85% dry matter basis. All analyses were performed using Genstat V14.1 (VSN International, Hemel Hempstead). Variates were $\log_{10}$ transformed to normalise the variance where necessary and back-transformed after analysis in some cases in order to facilitate comparison and interpretation by those in the industry who are more conversant with non-transformed arithmetic values. The conventional probability threshold (*P*) of <0.05 is taken as the threshold of statistical significance, apart from where indicated.

4. Results

4.1. Soil micronutrient survey

Originally, the NSI survey took samples every 5 km across England and Wales on a regular grid pattern in 1978-1982. This represents all soils, rather than just those under agricultural use. In order to make the two sets of samples more comparable, we used EU Corinne Land Use data to identify which samples in the NSI set have the land use “arable”. This reduced the number of samples in the large NSI to survey from 5,659 to 1,805.

The NSI samples will be referred to here as the “old” samples, and data for these was extracted from the Rothamsted Research NSI database. This database contains “total” analyses by aqua regia, and extractable analyses by EDTA. Because of the small losses of micronutrients, their removal in 30 years of cropping or leaching is likely to be very small and difficult to detect in the analyses of total metals (Table 2). Note that the mean and standard deviation were calculated on a log10 transformation of the data, but then back-transformed to give the geometric mean and standard deviation in Table 2. Total concentrations of Cu decreased by 1 mg/kg and Mn by almost 40 mg/kg, but Zn appears to increase by 5 mg/kg in the “new” soil samples.

Table 2. Summary statistics of the aqua regia concentrations of Cu, Mn and Zn from soil samples taken in 2009/10 and 1978-1982 (new and old respectively), all results in mg/kg.

Data	Geometric mean	Minimum	Maximum	Std. deviation
New Cu	17.4	3.37	84.1	1.65
Old Cu	18.3	3.80	933.2	1.66
New Mn	522.4	71.29	3419.8	2.02
Old Mn	561.1	23.01	4456.6	1.98
New Zn	85.5	16.41	297.9	1.59
Old Zn	80.2	13.00	1524.1	1.58

However, it is more important in a crop context to consider changes in the availability of these micronutrients rather than totals. Changes in availability are more likely to be detected in EDTA extracts, for example, if the bioavailable pool has been extensively utilised and not replenished, or if for example, farms use more or less lime than previously, the extractable amounts of Cu, Mn and Zn will increase or decrease respectively. This could occur even when the total concentrations do not change.

Box plots of the distributions of EDTA-extractable concentrations are non-parametric and therefore the data did not require normalisation. They show the inter-quartile range in the box, with the 50 percentile in the centre, and 25 and 75 percentiles at the bottom and top of the box respectively. The 10th and 90th percentiles are shown by the whiskers below and above the box and any outliers with smaller or larger concentrations then the whiskers are shown as points. The results for manganese show that the spread of the data was larger in the old samples, probably because there were more of them and the chances of finding outliers would be greater (Figure 2). The same was true for extractable Cu and Zn (Figure 3). In each case, there were many more outliers with high concentrations in the old data, most likely because more samples were taken, and the samples were taken systematically from all over England and Wales, perhaps including areas with high background concentrations of these elements.

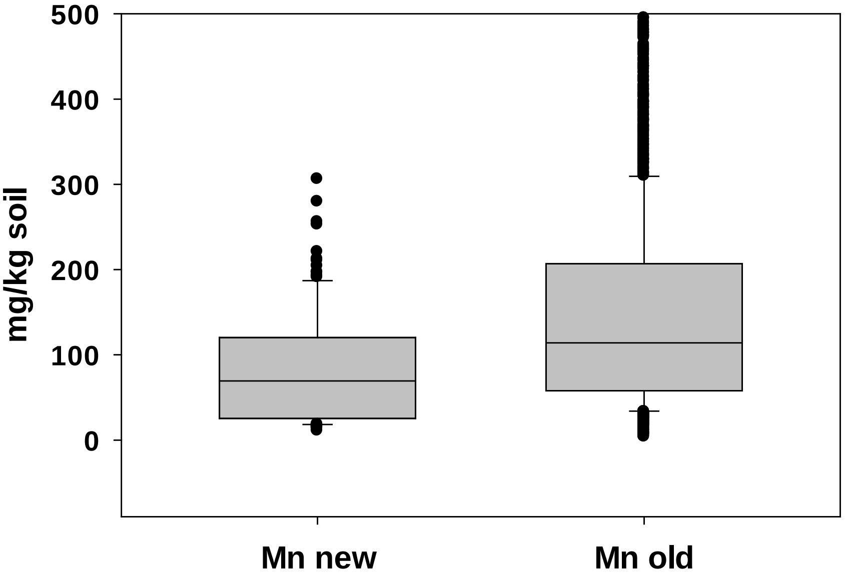


Figure 2. Concentrations of EDTA-extractable manganese in soil samples taken in 2009/10 and 1978-1982 (new and old respectively), all results in mg/kg.

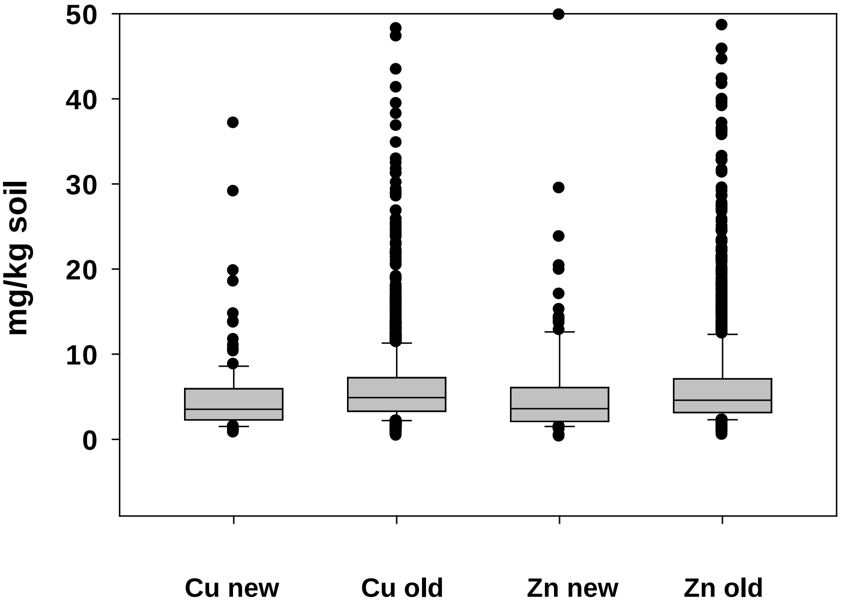


Figure 3. Concentrations of EDTA-extractable copper and zinc in soil samples taken in 2009/10 and 1978-1982 (new and old respectively), all results in mg/kg. Note that for clarity a few upper outliers have been removed for copper concentrations up to 111 and zinc up to 400 mg/kg.

Statistical distributions of new and old sample sets each of the three micronutrients were positively skewed, and so they were log-transformed prior to analysis. Table 3 shows the summary of the distributions of each data set after back-transformation. The maximum concentrations of every element differed between the two sample sets, which illustrates the point made above about the positive skew in the old data. The number of high results in the old data set would have a tendency to increase the geometric mean of that data set in comparison with the new data, which is clear for Mn (almost 50 mg/kg), but Cu and Zn only differ by just over 1 mg/kg.

One other important factor in considering these results is that no methods for extractable Mn in soil are considered useful (ADAS/MAFF, 1984), because in contrast to Cu and Zn, Mn availability in soil varies a great deal according to the moisture content of the soil.

Overall, the analysis of the new and old samples showed that as expected there was not much change in the total concentrations of the three micronutrients in soils taken about 40 years apart. It is important to point out that the samples were not taken in the same locations on each occasion. There was a trend of decreased EDTA-extractable concentrations, especially of Mn, over this time. However, there are a few issues that mean that this finding has to be qualified. One is that the surveys were different, as pointed out above, leading to more high outliers in the case of the old data set. Second is that the old survey systematically included all areas of England and Wales; and third that the soil Mn test is not used in practice, as it is not thought to reflect real differences in availability under field conditions.

Table 3. Summary statistics of the EDTA-extractable concentrations of Cu, Mn and Zn from soil samples taken in 2009/10 and 1978-1982 (new and old respectively), all results in mg/kg.

Data	**Geometric mean**	**Minimum**	**Maximum**	**Std. deviation**
New Cu	3.72	0.76	37.15	2.05
Old Cu	4.98	0.40	111.4	1.96
New Mn	60.5	11.0	693.4	2.40
Old Mn	107.6	4.0	1667.2	2.42
New Zn	3.78	0.30	54.8	2.37
Old Zn	5.00	0.50	400.0	2.05

4.2. Micronutrient response trials

The trial sites were chosen on soils that were likely to be deficient in micronutrients. The selection was based on the fact that soils that are light and sandy in texture, high in organic matter or calcareous are those in which there is either a low content of micronutrients (light texture) or that the micronutrients that are present are made less available by the soil conditions (high organic matter or high pH). Each site was sampled and the soil chemically analysed first for the pH and EDTA-extractable micronutrients and soil texture was visually assessed. On this basis, sites were chosen for the experiments. Many of the sites that were used for the field trials were below the lower quartile for EDTA-extractable Cu, Mn and Zn concentrations in the “new” soils data reported in Section 3.1. The fifteen sites are shown in Table 4. Where a location was used more than once, the trials were situated on different areas in each year. In total, there were 6 light soils, 6 organic soils and 3 calcareous soils. Winter wheat variety Solstice was sown in the autumn at all sites/years. Details of previous cropping are provided in Table 4.

Each of the sites was then characterised by measuring soil total Cu, Mn and Zn, total N, organic C, C/N ratio and Olsen P (Appendix Table 1). In addition, DTPA and ammonium nitrate extracts, and DGT analyses for Cu, Mn and Zn were performed on all sites (Appendix Tables 2-4).

Table 4. Dates, location, soil texture classification and previous cropping of the fifteen trial sites.

Year	Site	County	Soil classification	Previous crops (latest first)
2009/2010	Morley	Norfolk	loamy sand	Beans, W barley, W wheat
	Ely	Cambridgeshire	organic	Lettuce, Sugar beet, lettuce
	East Harling	Norfolk	loamy sand	Grass, W barley, W wheat
	Rothamsted	Hertfordshire	calcareous	W wheat, W beans, W wheat
	Woburn	Bedfordshire	sandy loam	W wheat, W oat, W wheat
2010/2011	Bardney	Lincolnshire	organic	Linseed, W wheat, Sugar beet
	Prickwillow	Cambridgeshire	organic	W wheat, lettuce, W wheat
	Stretham	Cambridgeshire	organic	Potato, W wheat, Sugar beet
	Rothamsted	Hertfordshire	calcareous	W beans, W wheat, W beans
	Woburn	Bedfordshire	sandy loam	W beans, W wheat, W wheat
2011/2012	Caythorpe	Lincolnshire	loamy sand	Sp barley, Sugar beet, W wheat
	Prickwillow	Cambridgeshire	organic	Lettuce, Wheat, Lettuce
	Waterbeach	Cambridgeshire	organic	Leek, Wheat, Sugar beet
	Rothamsted	Hertfordshire	calcareous	W beans, W wheat, W wheat
	Woburn	Bedfordshire	sandy loam	W wheat, potato, Sp barley

4.2.1. Visual symptoms

Visual symptoms characteristic of micronutrient deficiency were assessed in 2011 and 2012 on leaves at growth stage 33. Because visual symptoms are often transient, we had a note in the trial protocol to record symptoms at any visit if they were seen. None were observed at any site.

4.2.2. Grain yields

Grain yields at 85% dry matter and the F probability for the effects of the treatments from analysis of variance are shown for each site in Tables 5-7. Although high yields (9-11 t/ha) were obtained at some of the sites chosen, statistically significant effects of the treatment sprays on yield were found only in two cases; Zn (*P* = 0.04) on a loamy sand at Morley in 2010 and Cu in 2012 on a soil high in organic matter at Waterbeach near Cambridge (*P* = 0.058; note the slight relaxation of the *P* <0.05 rule: see below). The Zn spray increased yield by 0.27 t/ha, and Cu spray by almost 1.4 t/ha.

Table 5. Year 1 yields t/ha (2010 harvest)

Treatment	**Site**				
	Rothamsted (calcareous soil)	**Woburn (light soil)**	**East Harling (light soil)**	**Morley (light soil)**	**Ely (organic soil)**
Control	5.33	5.18	7.20	8.05	9.66
Copper	5.48	4.96	7.75	8.13	9.35
Manganese	5.48	5.06	7.73	7.92	9.96
Zinc	6.02	5.16	7.73	8.32	9.17
F Prob	0.358	0.741	0.999	0.043	0.218
Significance	*NS*	*NS*	*NS*	*	*NS*
l.s.d. (*P* <0.05)	0.79	0.48	1.47	0.26	0.84
% cv	10.7	7.1	14.8	2.5	6.7

Table 6. Year 2 yields t/ha (2011 harvest)

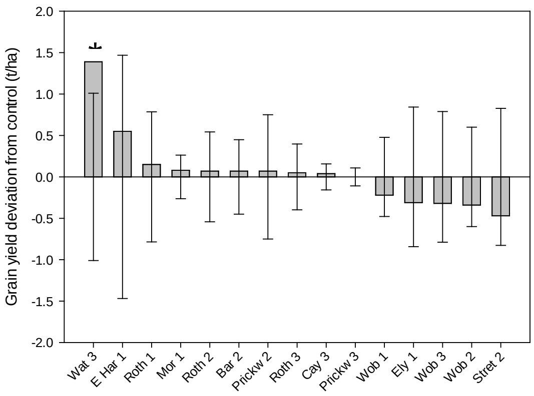
Treatment	**Site**				
	Rothamsted (calcareous soil)	**Woburn (light soil)**	**Bardney (organic soil)**	**Prickwillow (organic soil)**	**Stretham (organic soil)**
Control	11.29	8.09	6.57	10.52	7.21
Copper	11.36	7.75	6.64	10.59	6.74
Manganese	10.77	7.73	6.71	10.05	7.06
Zinc	11.46	7.67	6.86	10.67	7.09
F Prob	0.067	0.969	0.641	0.275	0.691
Significance	*NS*	*NS*	*NS*	*NS*	*NS*
l.s.d. (*P* <0.05)	0.54	0.60	0.45	0.75	0.83
% cv	3.7	5.8	5.1	5.4	8.8

Table 7. Year 3 yields t/ha (2012 harvest)

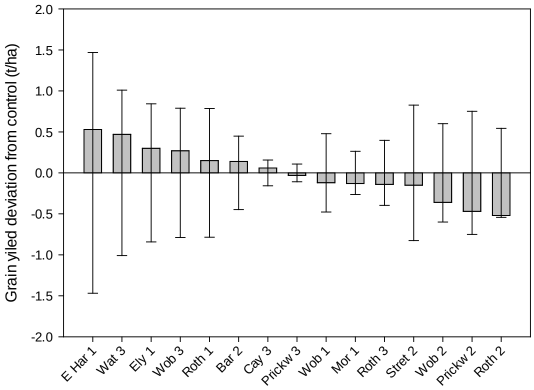
Treatment	**Site**				
	Rothamsted (calcareous soil)	**Woburn (light soil)**	**Caythorpe (light soil)**	**Prickwillow (organic soil)**	**Waterbeach (organic soil)**
Control	9.04	9.42	7.30	7.48	7.23
Copper	9.09	9.10	7.34	7.48	8.62
Manganese	8.90	9.69	7.36	7.45	7.70
Zinc	9.18	9.32	7.36	7.40	7.19
F Prob	0.425	0.401	0.948	0.357	0.058
Significance	*NS*	*NS*	*NS*	*NS*	*
l.s.d. (*P* <0.05)	0.40	0.80	0.16	0.11	1.01
% cv	3.3	6.4	1.6	1.1	10.0

To look for trends in effects on yield, the difference of each treatment mean from the control was calculated and presented graphically, along with the LSD from ANOVA for each experiment. For example, if many of the results were positive with respect to the control, but not statistically significant (at *P* <0.05), this would show as a trend, with most of the bars being positive. This was not the case for Cu, Mn or Zn (Figure 4). The lines and whiskers in the Figure are LSDs which indicate what magnitude of yield increase (t/ha) would be required in each particular experiment to be detected as “significant” by ANOVA. The LSDs at each site varied (from 0.11 – 1.47 t/ha), but were on average around 0.6 t/ha, meaning that this magnitude of change in yield would be necessary for a statistical difference to be detected.

Copper



Manganese



Zinc

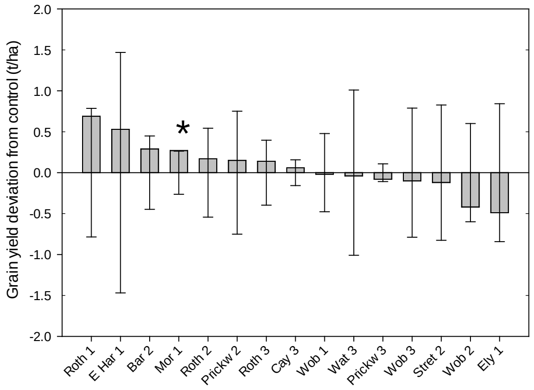


Figure 4. Mean yield of all treatments/years expressed as difference from control (untreated). Roth= Rothamsted; Wob = Woburn; E. Har = East Harling; Bar = Bardney; Prickw = Prickwillow; Stret = Stretham; Cay = Caythorpe; Wat = Waterbeach. Numbers refer to years: 1= 2010; 2=2011; 3= 2012. Asterisks indicate significant differences from control by analysis of variance (*P* <0.05). Black bars and asterisks = least significant difference (*P* <0.05).

Looking in detail at the raw data from the two significant positive responses, for Cu at Waterbeach in 2012, one block had a lower yield in all the treatments than the others (average yields were 8.05, 8.28, 6.57, 7.22 t/ha). This may be the reason for the large LSD value at this site, but in this case the yield response was big enough to be significant (+1.4 t/ha or 19.2%). This response may have been even more significant if there had not been a “bad block” in the experiment. Whereas, at Morley in 2010, the block averages for all treatments were 8.06, 7.85, 8.24 and 8.24, giving rise to a small LSD value (0.26) that was exceeded by the +0.27 t/ha (3.4%) increase in yield. This explains why relatively large or small increases can be designated as significant, depending on the magnitude of the error term in specific experiments. Across all the sites, the percentage yield increase needed to exceed the LSD values ranged from 1.5 – 20.4%, with an average of 8.3%. Micronutrient effects on yield, if any, may be within this range, and are therefore difficult to show statistically.

The exact experimental design is also important. We included 8 control plots as a design feature in these experiments. When one of the results from the two controls in each block is randomly omitted from the ANOVA, the P value for Morley increased from 0.043 to 0.074, and from 0.058 to 0.104 at Waterbeach. In both cases this would have made the yield effect not significant at $P = 0.05$ and these effects would not be detected. This emphasises the increased power of detection by employing double the number of replicates of control treatments, as used here.

Another aspect to consider is the probability level that is taken for assessing significance. Although $P < 0.05$ is conventionally used as the acceptable level of significance (this represents a 1 in 20 chance of being wrong due to random variation), many practitioners in the industry have asked, in view of the fact that relatively small yield increases are likely with micronutrients, what would happen if we relaxed this level of uncertainty, to $P < 0.10$. This is illustrated in Figure 5 and Table 8. These show that the size of the LSDs did not change very much, and no more positive responses become significant at $P < 0.10$. However, one response (for Mn at Rothamsted in 2011) becomes significantly negative. Sprays could possibly decrease yield if too much is used, or spraying is carried out in conditions that lead to scorch of the leaves, but we did not observe any visible symptoms of that in our experiments. At $P < 0.10$, there is more chance of being wrong in interpreting any response due to chance (one in ten). Relaxing this even further would lower these odds even further, and most likely draw in more of the “negative” responses as being “significant”. One would then have to accept more negative responses also, which is undesirable.

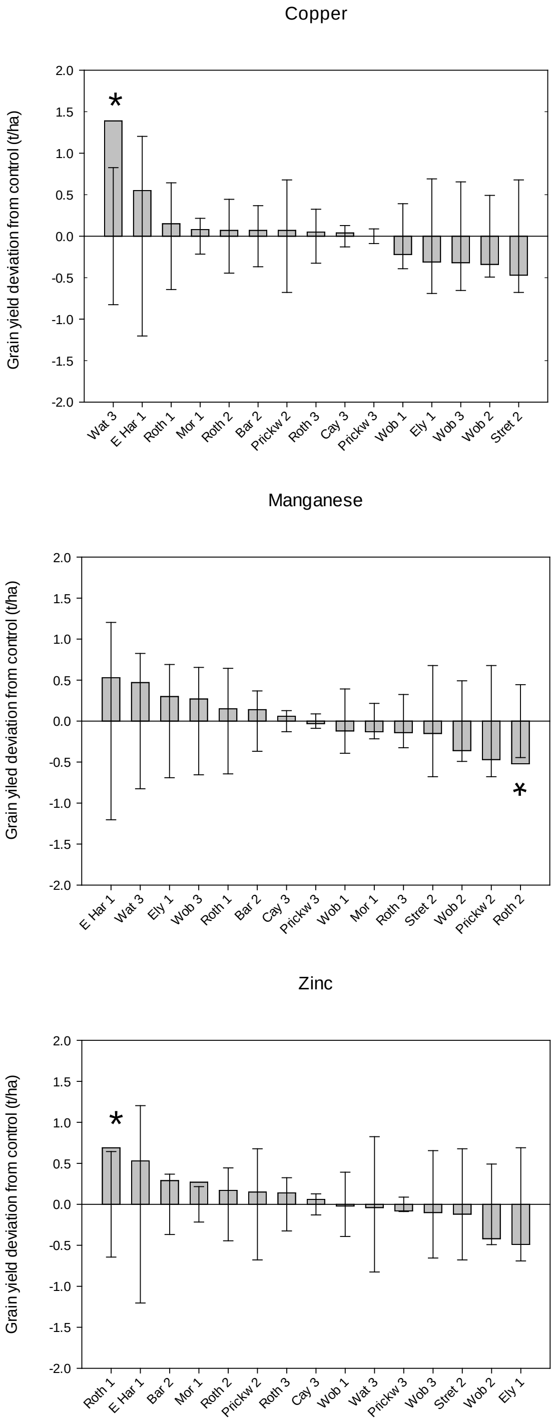


Figure 5. Mean yield of all treatments/years expressed as difference from control (untreated). Roth= Rothamsted; Wob = Woburn; E. Har = East Harling; Bar = Bardney; Prickw = Prickwillow; Stret = Stretham; Cay = Caythorpe; Wat = Waterbeach. Numbers refer to years: 1= 2010; 2=2011; 3= 2012. Asterisks indicate significant differences from control by analysis of variance (*P* <0.10). Black bars and asterisks = least significant difference (*P* <0.10).

Table 8. Summary of least significant differences in yield from ANOVAs for fifteen site/years in t/ha of grain.

Probability	*P* = 0.05	*P* = 0.10
minimum	0.11	0.09
maximum	1.47	1.20
average	0.63	0.52

Next, taking the data for all 15 sites years together, we conducted Restricted Maximum Likelihood (REML) analysis and found that, using all the data, calcareous soil tended to yield most and the light soil least, but this was not significant due to variation across sites/years in yields ("*P* soil" in Table 9). Also, the effects of spray treatments on yields was not significant across sites ("*P* soil*(control/treatment)" in Table 9), which is not surprising in that there were only two significant responses out of a possible 45 comparisons (15 sites x 3 micronutrients).

Table 9. REML analysis of grain yields, including data for all sites and all years.

Soil Type	Treatment				
	Control	Cu	Mn	Zn	s.e.m
Calcareous (3)	8.55	8.65	8.38	8.89	1.03-1.04
Light (6)	7.54	7.5	7.58	7.59	0.73
Organic (6)	8.11	8.2	8.17	8.06	0.73
P soil 0.69 *NS*	*P* soil*(control/treatment) 0.21 *NS*				

4.2.3. Soil EDTA analyses

EDTA extracts of soils have been used for judging the micronutrient status for at least 50 years, but the link between the results and deficiency in crops is hard establish (Roques et al, 2013), and the evidence in terms of crop yield responses for any particular critical values is very scant. Judging the analyses by the ADAS/MAFF (1984) criteria for likely Cu deficiency in crops (Table 10), two sites would be classed as "deficient" in 2010 (Morley and Rothamsted), one in 2011 (Rothamsted) and two in 2012 (Waterbeach and Rothamsted; Appendix Tables 2-4). However, taking Roques et al's (2013) critical value of 1.0 Cu mg/kg, only Waterbeach and Rothamsted in 2012 would be classed as deficient. Using the ADAS value, only 1 in 5 predictions were correct, but employing the lower Roques et al value, 1 in 2 was correct. This appears to justify use of the lower critical value for Cu (1.0 mg/kg), which is also the value given in the Fertiliser Manual (2010). However, it is worth noting that the

ADAS critical value for highly organic soils is 2.5 mg/kg, and the responsive site Waterbeach had only 0.79 mg/kg extractable Cu, whereas the other 5 organic soils has much higher Cu concentrations (Appendix Tables 2-4). Only Bardney with 2.65 mg/kg extractable Cu in 2011 was near to ADAS borderline for organic soils, which emphasises just how Cu-deficient the Waterbeach soil was, and how likely that the response in yield was due to lack of Cu.

Taking Alloway's (2008) 1 mg/kg critical value for EDTA-extractable Zn, the Morley soil would have been considered borderline for Zn in 2010 (Zn was 1.07 mg/kg), so a 1 mg/kg critical value is probably justified. The Fertiliser Manual (2010) gives a value for probable deficiency in susceptible crops (suggested to be top fruit and forest nursery stock) of <0.5 mg/kg and < 1.5 mg/kg for possible deficiency. The Manual states that Zn deficiency is likely on sandy soils with high pH and phosphate status, which is true for Morley, except that this site is not of high P status (Appendix Table 1). This work suggests that a 1 mg/kg critical value may be applicable to high-yielding wheat, even with a soil P index of 2. It also suggests that winter wheat should be included in the category of susceptible crops in the Fertiliser Manual.

Table 10. Criteria for EDTA soil extractable concentrations below which soils are considered "deficient" in micronutrients (based on ADAS/MAFF, 1984 and Alloway, 2008). See text for Roques et al's value for Cu.

Mn	Zn	Cu	
None	< 1	< 2.5	If soil organic matter >6%
		< 1.6	Other soils

Unfortunately, as stated in 2.3.1 above, no methods for extractable Mn in soil are considered useful (ADAS/MAFF, 1984).

It was hoped that many yield responses would be detected on these soils that were considered to be at risk of micronutrient deficiencies and that other soil extracts may have proven more useful than EDTA for predicting deficiencies across sites. However, there were only two yield responses and these were detected by EDTA (Zn-Morley 2010, Cu-Waterbeach 2012). The other extracts will not be discussed here, but mentioned in the Discussion (Section 4.2).

4.2.4. Leaf analyses

Young leaves were sampled at all sites at GS30 before the treatments were applied, which can help indicate whether the sites/crop would be classified as “deficient”. The criteria used for tissue samples were:

- Cu < 4 mg/kg in leaves, < 2 mg/kg in grain (Alloway B.J., 2008)
- Mn < 20 mg/kg in leaves and also grain (ADAS/MAFF, 1976; Alloway B.J., 2008)
- Zn < 20 mg/kg in leaves and also grain (Mortvedt J.J., et al, 1972; Scaife and Turner, 1983; Alloway B.J., 2008)

The number of sites/years that were either below or very close to these criteria were: three for Cu, two for Mn and four for Zn (Table 11). It is also of note that no site/elements had any deficiencies indicated by leaf analysis in 2010, two in 2011, and seven in 2012. Rothamsted and Woburn farms were used in all of these years, and the seasonal differences in weather were that 2010 was cold in January and February (soon before sampling took place), which was otherwise near the 30 year average, 2011 was cold in January but drier overall compared to average, and 2012 was cold in February but wettest overall (Appendix Tables 5-7). It is not known whether these features affect the concentrations of micronutrients in the leaves. It is also worth noting that there may be only small differences in Cu in tissues between healthy and deficient plants, and so tissue testing for Cu may be less useful than soil testing (Chalmers et al. 1999). The Fertiliser Manual (2010) does not suggest a critical value for leaf Cu, for Mn it gives 20 mg/kg and for Zn only 15 mg/kg. Using this lower Zn value, no sites would be flagged up as problematic, including the Morley site that in fact responded in yield to Zn sprays even though it had nearly 32 mg/kg Zn in leaves at the time of sampling.

Table 11. Mean micronutrient concentrations in leaf material (mg/kg) measured before treatments were applied. The standard error of the mean is given in parentheses. Results in red indicate those below or very close to the criteria given in Section 3.2.4.

Year	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn
2010	Rothamsted			Woburn			East Harling			Morley			Ely		
	(calcareous soil)			(light soil)			(light soil)			(light soil)			(organic soil)		
	12.97	91.27	26.76	12.19	29.16	29.65	10.86	29.14	39.00	10.21	78.89	31.66	12.23	42.41	50.23
	(0.76)	(9.12)	(1.40)	(0.76)	(0.93)	(0.27)	(0.53)	(0.96)	(2.51)	(0.35)	(5.13)	(1.54)	(0.62)	(3.56)	(1.44)
2011	Rothamsted			Woburn			Bardney			Prickwillow			Stretham		
	(calcareous soil)			(light soil)			(organic soil)			(organic soil)			(organic soil)		
	7.42	75.41	20.94	5.91	32.61	17.43	4.07	39.07	32.81	7.00	26.59	29.30	5.72	66.90	40.55
	(0.07)	(2.47)	(0.91)	(0.10)	(0.88)	(0.55)	(0.17)	(2.53)	(0.39)	(0.09)	(0.76)	(1.11)	(0.07)	(4.99)	(1.06)
2012	Rothamsted			Woburn			Caythorpe			Prickwillow			Waterbeach		
	(calcareous soil)			(light soil)			(light soil)			(organic soil)			(organic soil)		
	6.41	54.31	18.73	4.64	8.55	17.34	3.68	44.13	18.84	7.52	28.85	30.02	3.91	13.08	24.84
	(0.20)	(0.18)	(0.74)	(0.18)	(0.71)	(0.31)	(0.11)	(1.14)	(0.91)	(0.77)	(9.05)	(0.68)	(0.28)	(1.66)	(0.83)

4.2.5. Grain analyses

The concentrations of Cu, Mn and Zn were measured in all grain samples after harvest. The results are important for three reasons: 1) they can be used to indicate if the crop would be classified as “deficient” using the criteria given above, 2) if concentrations increase, these can act as an indication of the success of the treatments spayed on the leaves, in other words whether the plant and grain has responded to the additions and 3) there is interest internationally (see HarvestZinc, 2012) in boosting the concentrations of micronutrients in grain for animal and human nutrition, and it is useful to know if early foliar sprays can contribute to this biofortification.

The values given below indicate that a total of 1 site would be classified as “low” in grain Cu (Waterbeach, 2012) and this helps to confirm that the plants were severely deficient in Cu at that site (Tables 12-14). Seven were “deficient” in Mn but did not respond in either yield or Mn concentration in grain, apart from Prickwillow in 2011, which increased by only 1.5 mg/kg in grain in the Mn treatment (Tables 12-14). This argues that Mn is not very mobile from leaves to grain, which is supported by Rengel (1999). Four sites were classed as “deficient” for Zn, three of which had light textured soils, out of six of the sites with this type of soil (Tables 12-14). One of these, with the lowest grain Zn concentration of all sites was the Zn responsive site Morley (2010). This had 15.52 mg/kg Zn in grain from the control treatment, which would not be classified as “deficient” if the 15 mg/kg criterion is used. Again, this may justify using 20 mg/kg as a cut-off for grain Zn.

Although these experiments were not set up to create different yields within each site, there was no indication across sites/years of so-called “growth-dilution” of Zn concentrations in grain (Gooding et al, 2012). For example, on the calcareous soil at Rothamsted, Zn concentrations were around 17, 25 and 26 mg/kg in 2010, 2011 and 2012 when yields were 5.3, 11.3 and 9.0 t/ha respectively. In 2010 this was a second wheat (Table 4), but it is difficult to disentangle the potential effects that this may have on roots from factors related to weather and soil (site). At Woburn, second wheats were grown in 2010 and 2012, but the accompanying yields were 5.18 t/ha and 9.42 t/ha and Zn concentrations in grain were 16.94 mg/kg and 21.37 mg/kg. However, 2011 at Woburn was a first wheat and yielded 8.09 t/ha and had 17.77 mg/kg in grain. It is notable that the soils high in organic matter gave amongst the highest Zn concentrations in grain, 29-43 mg/kg Zn (Tables 12-14).

Table 12. Year 1 micronutrient concentrations in harvested grain (2010 harvest). Results in red indicate those below or very close to the criteria given in Section 3.2.4.

	Site														
	Rothamsted (calcareous soil)			Woburn (light soil)			East Harling (light soil)			Morley (light soil)			Ely (organic soil)		
Treatment	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
Control	3.24	30.92	17.83	3.61	16.18	16.94	3.78	17.82	22.32	3.32	28.53	15.52	3.92	22.52	33.47
Cu	3.15	30.40	17.30	3.48	15.63	16.64	3.62	18.44	21.59	3.45	27.42	15.18	3.88	22.91	34.34
Manganese	2.88	30.68	17.88	3.31	14.76	16.86	3.62	17.11	20.62	3.13	27.04	14.30	4.02	25.10	33.07
Zinc	3.05	30.86	17.84	3.48	15.83	17.63	3.63	18.38	24.52	3.32	28.06	16.70	3.95	21.55	35.48
F Prob	0.294	0.874	0.515	0.464	0.629	0.161	0.994	0.181	0.004	0.054	0.662	0.054	0.719	0.119	0.250
Significance	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
l.s.d. (P <0.05)	0.31	1.66	1.02	0.29	2.17	0.95	0.22	1.43	1.83	0.22	2.10	1.68	0.33	2.99	2.57
% cv	7.5	4.1	4.4	6.2	10.5	4.2	4.4	6.0	6.2	5.1	5.7	8.2	6.3	9.8	5.7

Table 13. Year 2 micronutrient concentrations in harvested grain (2011 harvest). Results in red indicate those below or very close to the criteria given in Section 3.2.4.

	Site														
	Rothamsted (calcareous soil)			Woburn (light soil)			Bardney (organic soil)			Prickwillow (organic soil)			Stretham (organic soil)		
Treatment	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
Control	3.91	32.17	25.53	3.11	16.65	17.77	2.56	30.24	43.13	3.33	13.64	29.18	4.15	25.22	34.35
Cu	3.83	31.60	24.79	3.19	16.78	17.75	2.57	29.53	42.02	3.42	14.40	28.42	4.23	23.17	35.67
Manganese	3.93	31.60	25.49	3.17	17.21	17.82	2.66	30.42	44.51	3.36	15.15	28.33	4.31	25.32	35.79
Zinc	3.76	32.28	25.56	3.16	16.56	19.60	2.50	31.40	43.83	3.10	13.11	30.38	4.21	25.07	36.10
F Prob	0.291	0.839	0.705	0.962	0.647	0.056	0.348	0.713	0.471	0.076	0.011	0.144	0.828	0.163	0.951
Significance	*NS*	*NS*	*NS*	*NS*	*NS*	*	*NS*	*NS*	*NS*	*NS*	**	*NS*	*NS*	*NS*	*NS*
l.s.d. (*P* <0.05)	0.19	2.45	1.89	0.20	1.30	1.45	0.19	4.18	3.80	0.25	1.06	2.04	0.32	2.15	2.67
% cv	3.8	5.8	5.6	4.9	5.9	6.0	5.5	10.4	6.6	5.7	5.7	5.3	5.8	6.6	5.7

Table 14. Year 3 micronutrient concentrations in harvested grain (2012 harvest). Results in red indicate those below or very close to the criteria given in Section 3.2.4.

	Site														
	Rothamsted (calcareous soil)			Woburn (light soil)			Caythorpe (light soil)			Prickwillow (organic soil)			Waterbeach (organic soil)		
Treatment	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
Control	3.39	36.49	26.26	2.03	11.15	21.37	2.50	27.70	20.10	2.94	18.32	37.65	1.75	10.94	40.96
Cu	3.28	35.50	23.96	2.24	11.26	30.30	2.48	28.18	20.41	3.05	19.12	35.96	1.86	11.28	38.72
Manganese	3.32	36.23	25.39	2.08	12.75	20.18	2.45	28.73	20.75	2.94	17.77	39.71	1.80	11.32	38.74
Zinc	3.45	36.98	30.68	2.05	10.31	26.84	2.36	27.84	21.49	2.83	18.14	35.55	1.84	13.74	40.77
F Prob	0.292	0.516	<0.001	0.201	0.176	0.002	0.535	0.463	0.311	0.238	0.493	0.040	0.921	0.140	0.669
Significance	*NS*	*NS*	***	*NS*	*NS*	**	*NS*	*NS*	*NS*	*NS*	*NS*	*	*NS*	*NS*	*NS*
l.s.d. (*P* <0.05)	0.20	2.34	1.87	0.21	2.30	3.01	0.20	1.31	1.29	0.23	2.14	2.98	0.27	2.46	4.85
% cv	4.5	4.9	5.4	7.5	15.4	10.4	6.0	3.5	4.7	6.0	8.8	6.0	11.4	15.9	9.2

ANOVA of the effects of the treatments on grain concentrations (Tables 12-14) showed that only the Zn treatment boosted the concentration of Zn in grain on the light soil at Morley in 2010 (*P* =0.004), and on another light site at Woburn in 2011 (*P* =0.056) and 2012 (*P* =0.002), and on one calcareous site at Rothamsted in 2012 (*P* <0.001). Mn spray increased Mn in grain at Prickwillow in 2011 (*P* <0.01). Zn spray significantly increased grain Cu at Woburn in 2012 and Mn spray increased Zn in grain at Prickwillow in 2012; these effects on other elements were either indirect ones or by occurred by chance. Correspondence between “low” micronutrient classification of grain and the significant and element-specific responses in terms of concentration in grain to spray treatments was low. For example, 5 site/element combinations were classified as “low” in 2010 but none of these responded to the fertiliser by boosting the “low” grain concentrations (Table 7). In 2011 there were two sites where grain concentrations responded to Zn and Mn respectively and were classed as “low”. Finally, 4 site/element combinations classified as “low” in 2012 did not respond significantly, whilst two that were not classified as “low” (Rothamsted and Woburn) responded specifically in grain concentrations to the Zn spray applied.

Table 15. REML analysis of micronutrients present in grain at harvest, across all years.

Soil Type	Predicted element	Treatment				
		Control	Cu	Mn	Zn	s.e.m
Calcareous (3)	Cu	3.51	3.42	3.37	3.42	0.43-0.44
Organic (6)	Cu	3.13	3.19	3.20	3.09	0.37
Light (6)	Cu	3.04	3.06	2.94	2.98	0.37
P soil 0.38 *NS*		*P* soil*(control/treatment) 0.21 *NS*				
Calcareous (3)	Mn	33.19	32.50	32.83	33.37	3.73-3.75
Organic (6)	Mn	20.15	20.07	20.85	20.50	2.64-2.65
Light (6)	Mn	19.67	19.62	19.60	19.50	2.64-2.65
P soil 0.03**		*P* soil*(control/treatment) 0.64 *NS*				
Calcareous (3)	Zn	23.00	22.02	22.92	24.69	2.44-2.48
Organic (6)	Zn	36.30	35.69	36.53	36.86	1.86-1.88
Light (6)	Zn	19.16	18.80	18.58	21.29	1.86-1.88
P soil <0.001***		*P* soil*(control/treatment) 0.05*				

REML analysis of all 15 site/years by soil texture type confirmed no overall significant effect of soil type on Cu in grain. However, Mn was lowest in organic and light soils (*P* = 0.03), with calcareous soils highest (Table 15). There was no overall effect of treatment on grain Cu and

Mn when all data were pooled. However, for Zn the calcareous and light sites gave the lowest grain Zn concentrations, and both types of soil showed increases in grain Zn over control in the Zn concentrations in the Zn treatments (*P* <0.05). But the overall increase comparing +Zn with Control across all site/years was small (c. 1.7 – 2.1 mg Zn/kg in grain).

5. Discussion

5.1. Soil micronutrient survey

Although the aqua regia “total” concentrations of Cu, Mn and Zn differed slightly between our samples taken in 2009/10 and the NSI samples in 1978-1982, the differences were not large. The geometric mean of the sample results for Cu were lower in 2009/10 by less than 1 mg/kg and Mn by nearly 40 mg/kg, but for Zn the new samples were higher by almost 5 mg/kg (Table 2). Due to pollution control and changes in industries, there have been decreases in atmospheric emissions of these elements between 1980 and 2010 of about a factor of two for Zn and Cu. Manganese emission data have only been collected for the last 10 years, but these also show a decline (Figure 6). UK emissions follow the order Zn > Cu > Mn. However, due to the long residence times of metals in soils, decreases are as yet difficult to measure (Emmet et al, 2010). Indeed, in the case of Cu only, the Review of Transboundary Air Pollution (RoTAP, 2012) report gives a map of the total deposition in 2008 that shows that most of lowland UK receives from 5 – 50 g/ha/yr Cu from atmospheric sources. Although all of the Cu deposited may not be in a bioavailable form, this input alone is a similar order of magnitude as the offtake of Cu in grain of cereal crops cereal crops, which is estimated at 30 – 90 g/ha/yr for a 10 t crop (Roques et al, 2013). In addition to this, some farms/fields may gain much greater amounts Cu from additions such as farm manures, sewage sludge, composts or other recycled materials (Nicholson et al, 2003). In the Countryside Survey, Emmett et al (2010) reported a general increase in Cu concentrations in soils sampled nationally in 1998 and 2007, although detailed breakdown of the results by land use actually shows no change in arable and horticultural soils. For Zn, arable and horticultural soils showed a slight decrease (average of 90.1 to 84.4 mg/kg). Manganese was only measured in 2007 in the Countryside Survey.

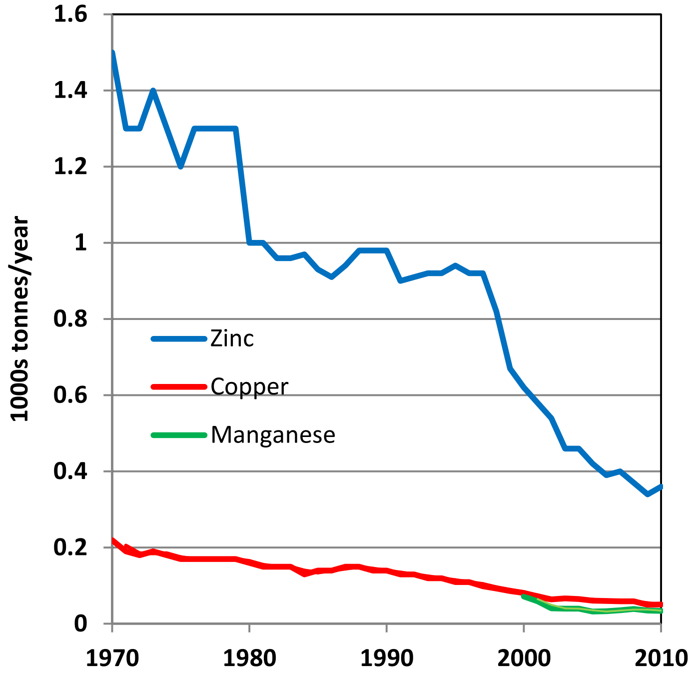


Figure 6. UK emissions of Zn, Cu and Mn (http://naei.defra.gov.uk/)

It is more relevant to the availability of micronutrients to crops to measure an extract, such as EDTA, that was also measured in 1979-1982 in the NSI. These have the same issues related to detecting changes as aqua regia values (see Section 2.3.1), but the results show only a decrease of just over 1 mg/kg for Cu and Zn (Table 3). For Mn the difference is almost 50 mg/kg, but EDTA extracts for Mn are affected by factors such as the moisture content of the soil and are not related to crop uptake. It is unlikely that the differences for Cu and Zn are biologically significant. In addition, the changes may be due to random sampling error, and could only be made more comparable by sampling exactly the same sites in 2009/10 as in 1978-1982. There is uncertainty in these results, but what is known is that there has not been a large change in either total or extractable Cu, Mn or Zn in arable soils.

5.2. Micronutrient response trials

The spray treatments with Cu, Mn or Zn in the 15 site/years mean that there were a total of 45 possible responses in the trials. Out of these we detected two yield responses, one each for Cu and Zn. Careful examination of the responses and changing the usual probability level from $P < 0.05$ to $P < 0.10$ did not change these results, except that one apparent negative yield effect with Mn is suspected if $P < 0.10$ is applied.

For Cu, comparing this with the data reviewed by Roques et al, (2013), they reported 9 out of 60 UK trials with winter wheat responded in yield (15%), against our 1 out of 15 (7%). However, scrutiny of their reported data show that 4 of the responses they report were obtained between 1953 and 1972, i.e. they may be old enough to pre-date good advisory practice. In fact, only 1 of the 20 (5%) winter wheat trials they summarised as performed since 2005 responded to Cu (the Waterbeach result from this study). The latter was during a period when wheat yields were likely to be higher, and soil Cu may have since been enhanced in many previously Cu-deficient areas by many years of agricultural applications of sprays and perhaps soil Cu dressings which remain effective for many seasons (Roques et al, 2013). The 1 in 20 figure is far below the total of 61 out of 113 (53%) responses to Cu that Roques et al (2013) reported for all cereals in UK trials. Only one third of the cereal trials they reported were with winter wheat. Most of the other crops were spring wheat, spring barley and oats; the responses of these crops may be different to winter wheat.

The Cu response was observed in 2012, which was a relatively cool and wet season (Appendix Tables 5-7). Under these conditions, fungal diseases could be expected, but these were not assessed in the project. The product used contained Cu oxychloride, which is used on crops other than wheat as a fungicide. A robust regime of fungicides was applied at all sites. It is possible though that the increase in yield that was seen specifically in the Cu treatment at Waterbeach (+1.4 t/ha) was due to a fungicidal effect of Cu oxychloride. However, the rate of use as a fungicide is in fact about 20 times greater than used here. Also, extra evidence that this was a severe shortage of Cu in the crop at this site is given by the fact that the soil EDTA-extractable Cu was extremely low (0.79 mg/kg) compared to all other 14 site/years (average 3.31 mg/kg) and the grain Cu concentration was only 1.81 mg/kg averaged across all treatments at Waterbeach (and 1.75 mg/kg in the untreated control), compared to the average at other sites of 3.31 mg/kg.

We recorded no yield responses due to Mn, against 28 out of 79 trials (35%) reported by Roques et al, (2013) in all UK cereals trials. However, only 2 out of 20 trials they summarised for all cereals since 2005 responded to Mn, and 0 out of 16 winter wheat trials. The latter result is very similar to our trials.

For Zn we recorded one responsive trial out of 15 (7%), compared with 6 out of 36 in all UK cereals trials (17%) reported by Roques et al (2013). In trials since 2005, the latter authors report that 3 out of 19 or 16% (two winter wheat and one winter barley) of trials responded to Zn. It appears that the responsiveness in other trials to Zn in the UK since 2005 is slightly greater than in our trials.

Critical values of micronutrients in soils or plants can be useful where applicable to help predict where micronutrient applications are needed. Not enough responses occurred in this study to help calibrate these values, but it is possible to compare the existing or suggested values with our results. On this basis, the older ADAS values for EDTA-extractable Cu of <2.5 mg/kg for organic soils and < 1.6 for other soils do not appear justified, as 5 sites would be classed as "deficient" out of the 15 by soil analysis, but of these only one responded in yield. That site, Waterbeach, had a soil value of 0.79 mg/kg, but the next lowest site was Rothamsted with a soil of 1.0 mg/kg, and it did not respond. This appears to justify the lower EDTA-extractable value for Cu of 1.0 mg/kg that appears in the Fertiliser Manual (2010). For Zn, the Fertiliser Manual suggests 0.5 - 1.5 mg/kg EDTA-extractable for "susceptible crops" and our data support 1.0 mg/kg as a critical value for high-yielding wheat.

Other methods for micronutrient analyses were performed on all the soil samples taken before the spray treatments (Appendix Tables 2-4). Because there were not many yields responses, detailed analysis of whether yields fitted one method better than another was not possible. However, simple comparison of the results for the responsive and non-responsive sites with those for different extracts was done, with the aim of finding out which extracts if any were specific to those responses. For Cu, EDTA was correct in that Waterbeach gave the lowest soil value (0.79 mg/kg) than any other site and responded in yield to Cu. For DTPA, Cu was lowest at Bardney, and the same at Caythorpe as the responsive site. The DGT technique stood out by showing by far the lowest Cu value at Waterbeach. For Zn, EDTA correctly gave the lowest value for Morley which responded in yield to Zn. DTPA correctly showed the lowest value (0.9 mg/kg) at Morley, but Caythorpe was close to this (1.0 mg/kg) and did not respond. For DGT, as many as five sites showed lower Zn values than the responsive site. From this rather limited response information, it seems that EDTA and DGT are useful for Cu, and for Zn EDTA was more predictive than DTPA and particularly DGT. Ammonium nitrate was also used as an extractant, but even with sensitive ICP-MS analysis, the concentrations were too low to be reliable.

It is often thought that because of problems with a lack of availability of nutrients in the soil to the crop due to physical, chemical and even crops factors (e.g. poor rooting), that analysis of tissue samples may be more indicative of the bioavailability of micronutrients at particular sites. Done early enough, on young green tissue, it enables enough lead time for fertilisers to be included in sprays. Leaf analysis appears to be problematic, however, as it predicted as many as 9 site/element combinations as below or very close to critical values (Section 3.2.4), and yet only one of these responded in yield to micronutrient applications (to Cu). A total of 8 “false positives” therefore existed. The Cu-responsive site was Waterbeach with 3.91 mg/kg average leaf Cu, just under the 4 mg/kg suggested critical value. However, this site responded to Cu with a large increase in yield and had a particularly low grain Cu concentration. Caythorpe and Bardney did not respond, despite leaf concentrations of 3.68 and 4.07 respectively. The Morley site responded to Zn even though it had 32 mg/kg Zn in leaves sampled in spring, against a critical concentration of 20 mg/kg (a “false negative” result). It may not, therefore be useful to drop the critical value to 15 mg/kg as given in the Fertiliser Manual. Put against this are three sites that had 17.3, 17.3 and 18.7 mg/kg EDTA extractable Zn (Woburn 2011, 2012, Rothamsted 2012) and a Zn response was not detected. But if the 15 mg/kg critical value is used, none of the sites would have been flagged as “deficient”. This illustrates the difficulties of calibrating soil analyses values against variable responses from field trials.

Another important aspect of field trials is their ability to detect yield increases. For micronutrient responses, the magnitude appears to be relatively small (Roques et al, 2013). We have shown that with this trial design including double the number of replicates in the control treatments, on average a yield increase of 0.6 t/ha (8.3%) is needed before an effect is likely to be statistically significant at $P < 0.05$. This varies between sites, from 0.11 to 1.47 t/ha across our trials, due to many factors including soil variation, crop establishment and so on. So, the ability to identify, say 0.2 – 0.3 t/ha increase is challenging, if that is a realistic crop response to micronutrients. It may however, be very economic if that response is obtained, as the cost of micronutrient spays may be recouped with less than 0.1 t/ha gain in yield (Roques et al, 2013). There is therefore a disparity between the ability to detect yield responses with standard experimental designs and the cost/benefit of even small yield increases. An example of a response that was almost missed was Morley in 2012 that responded to Zn spray with 0.27 t/ha increase in yield ($P = 0.04$; Table 5). This significant effect was only detected when the results were re-analysed with the 8 control plots contrasted with the fourfold replicated micronutrient treatments. A yield effect of this size and economic value would have been missed if the background variation at this site had not been low. We have also shown that increasing the probability threshold from 0.05 to 0.10 creates more problems with potential “false positive” significant decreases, where yields appear smaller than controls, but it is important to remember that this may be due to random variation. For this reason it may not be acceptable to increase the number of these by relaxing the probability threshold (Figure 5, Table 8).

Zinc deficiency affects around one-third of the world's population, (Hotz and Brown, 2004,) particularly children. Lack of Zn affects brain function and mental development, depresses the immune system increasing susceptibility to infectious diseases and causes delays in physical development. There is therefore international interest in increasing the Zn concentrations of staple foodstuffs, especially wheat and rice. A programme called HarvestZinc (2012) has a target for biofortifiying Zn in wheat grain from the average 25 mg/kg to 33 mg/kg (HarvestZinc, 2012) and it is therefore of interest to compare the effect of the spays used here on grain Zn. Control grain Zn on grouped calcareous or light sites averaged 23 and 19.16 mg/kg respectively, and where Zn spray was applied these increased to 24.69 and 21.29 mg/kg by REML analysis across years and sites (Table 15). So, our "low" Zn sites started off with lower than the world average Zn concentration that HarvestZinc organisation assume, and only increased by 1.7-2.1 mg/kg, rather than 8 mg/kg. However, where our crops at individual sites responded significantly and specifically to Zn sprays, the average increases were 2.20, 1.83, 4.42 and 5.47 mg/kg at Morley in 20120, Woburn in 2011 and 2012, and Rothamsted in 2012 (Tables 12 - 14). To increase grain Zn even more may require later sprays or a combination of sprays and soil applications (Cakmak et al, 2010; McGrath et al, 2012). No specific responses in grain micronutrients were found in 2010 and none for Cu and Mn, apart from Mn in the highly organic Prickwillow site (Table 13).

Although there was no evidence of "growth dilution" of Zn concentrations in grain (Section 3.2.5), this is in fact a complex interaction between the amount of N applied and yield (Gooding at al, 2012). In each of these experiments, a single amount of N was applied, but what seems clear is that modern high-yielding varieties already have decreased concentrations of Zn (Fan et al, 2008; Gooding et al, 2012). This dilution, due to increased yield, has been observed for Cu and Mn (Fan et al, 2008; Gooding et al, 2012). However, in our results there is no sign that just because micronutrient concentrations are low in grain due to high yield that the crop responds in yield to these micronutrient sprays.

5.3. Conclusions

The objectives of this study pose the following questions and related conclusions:

Are soils changing in their Cu, Mn and Zn status?

Small decreases in EDTA-extractable concentrations of Cu, and Zn in soils over 30 years can be seen in the data. (c. 1 mg/kg), but this is not thought to be biologically significant. But EDTA-extractable Mn decreased by 50 mg/kg in this period. However, EDTA-extractable Mn is not used for diagnostic purposes. Total concentrations of Cu and Mn decreased, but total

Zn increased during this period. There is uncertainty in these apparent changes, as the same geographical locations were not measured at each occasion.

Do high yielding wheat varieties respond more to Cu, Mn and Zn?

Responses of winter wheat to micronutrients in terms of yield are rare and there were two (Cu and Zn) in this set of 15 site/years of experiments, even though they were chosen to be on soils where farmers are likely to apply micronutrients. However, the detailed data show no indication that the higher yielding years/sites responded more to micronutrients. The size of the yield responses observed were 0.27 (Zn) and 1.39 (Cu) t/ha.

Does the small size of yield responses for micronutrients make them difficult to detect?

Yes, as the least significant differences in yield (P <0.05) in these 15 experiments ranged from 0.11 – 1.47 t/ha (average 0.6 t/ha) depending on the site. In these trials, an average yield response of 8.3% was needed to make the effect statistically significant at P =0.05. The 0.27 t/ha increase in grain yield due to Zn was only detectable due to a particular experimental design and analysis used throughout which incorporates double control (untreated) plots, and because that particular site had a low variability. It is possible that economically useful responses (cost of micronutrient fertiliser/value of grain) are not detected using standard experimental plot designs. We observed both positive and negative economic impacts from using these materials, although most were not significant. Increasing the significance threshold to P <0.10 increases the chances of categorising negative yield responses as significant, and did not reveal any more significant yield increases in our experiments. One other way to look at whether there were more true responses (even if not statistically significant) is to simply look at yields in each treatment relative to control, and to determine if there were more positive than negative responses in our data. However, there was no evidence that this was the case in the 15 trials (Figure 4).

Does soil and leaf tissue analysis help predict when yield will be affected?

Soil analysis appears to result in more correct recommendations for Cu and Zn than leaf analysis in these particular experiments, but only if a 1 mg/kg EDTA-extractable critical concentration is adopted for both micronutrients. Leaf analysis at GS30 appears to result in more false positives, where low leaf analysis results do not always mean that there will be a detectable yield benefit from micronutrient applications. The critical leaf Mn concentration suggested in the Fertiliser Manual is 20 mg/kg and there were two sites below this, but they did not respond to Mn applications. Similarly, it recommends a critical value of 15 mg/kg for leaf Zn but no sites were below this in our results; indeed the responsive site had leaf analysis values that were more than double the critical value. Our limited response data do not suggest that leaf analysis in spring is useful.

Modern high-yielding wheats may have decreased micronutrient concentrations: is biofortification of grain Cu, Mn and Zn concentrations possible using standard sprays, for the benefit of animal and human health and nutrition?
Only in the case of Zn, but not reliably, and in the magnitude of increase that is necessary to boost nutrition. For Zn, a combination of soil and late foliar sprays may be needed to boost grain Zn concentrations.

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Appendices

Appendix Table 1. Soil characterisation data for all sites and all years, s.e.m = standard error of the mean. Olsen P is in mg/kg dry soil.

Site	Year	pH		Tot % N		Org C %		% Inorg C		C/N ratio		Olsen P		% Sand	% Silt	% Clay	Soil texture class
		Mean	s.e.m	Mean	s.e.m	Mean	s.e.m	Mean	s.e.m	Mean	s.e.m	Mean	s.e.m				
Rothamsted	2009/10	7.8	0.03	0.2	0.00	2.4	0.07	3.6	0.13	10.9	0.33	25.7	2.43	10	51	40	Silty clay
Woburn		6.5	0.00	0.1	0.09	1.1	0.02	0.0	0.00	12.9	0.32	51.3	1.12	70	20	10	Sandy loam
East Harling		6.4	0.15	0.2	0.00	1.8	0.06	0.0	0.00	11.2	0.33	38.1	2.46	80	9	11	Loamy sand
Morley		7.5	0.02	0.1	0.00	0.9	0.04	0.0	0.01	10.3	0.29	23.9	0.53	73	15	12	Loamy sand
Ely		7.4	0.08	0.9	0.07	13.7	1.02	0.3	0.09	14.5	0.06	54.4	1.89	5	52	45	Silty clay
Rothamsted	2010/11	7.7	0.10	0.2	0.01	2.2	0.08	2.9	0.55	9.5	0.19	73.9	0.82	13	42	45	Clay
Woburn		7.0	0.05	0.1	0.00	1.0	0.03	0.0	0.00	10.9	0.07	37.0	0.14	63	26	11	Sandy loam
Bardney		8.1	0.07	1.9	0.04	28.9	0.90	0.0	0.00	14.8	0.17	22.7	2.06	11	31	57	Clay
Prickwillow		5.9	0.10	1.0	0.09	14.6	1.50	0.4	0.10	14.3	0.16	38.2	2.47	7	48	45	Silty clay
Stretham		6.5	0.10	1.3	0.03	16.4	0.45	0.0	0.00	12.8	0.09	27.2	2.30	44	20	36	Clay loam
Rothamsted	2011/12	7.8	0.02	0.2	0.00	2.1	0.03	3.7	0.19	9.2	0.11	27.6	1.83	10	51	39	Silty clay
Woburn		7.0	0.05	0.1	0.00	1.1	0.01	0.0	0.01	11.0	0.19	74.5	2.75	87	6	7	Sand
Caythorpe		7.9	0.02	0.2	0.00	1.4	0.03	0.4	0.06	9.1	0.07	24.5	0.66	74	11	15	Sandy loam
Prickwillow		7.4	0.03	1.2	0.10	16.9	1.57	0.3	0.04	13.8	0.16	65.6	7.61	4	47	49	Silty clay
Waterbeach		7.2	0.07	2.0	0.05	28.1	0.83	0.9	0.41	14.4	0.13	26.0	1.77	19	61	20	Silty clay loam

Appendix Table 2. Year 1 (2009/10) Mean micronutrient concentrations in soil and leaf material measured pre-experimental treatment applications by total acid digestion (Total) or using different soil extraction methods. The standard error of the mean is given in parentheses. Values in red are below or very close to the criteria used in section 3.2.3 for deciding whether soils are likely to respond to micronutrient additions.

Method	Site														
	Rothamsted (calcareous soil)			Woburn (light soil)			East Harling (light soil)			Morley (light soil)			Ely (organic soil)		
	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn
Total (soil) mg/kg	19.80	1176.00	106.90	10.32	369.50	62.73	10.91	98.85	71.08	10.43	313.90	57.30	39.87	606.10	83.77
	(0.79)	(20.50)	(2.44)	(0.82)	(12.89)	(1.99)	(0.26)	(2.23)	(1.15)	(0.35)	(19.76)	(1.07)	(2.03)	(13.30)	(2.67)
EDTA (soil) mg/kg	1.56	9.68	2.30	2.78	49.43	1.82	3.63	22.00	12.43	1.23	27.66	1.07	9.28	49.71	5.98
	(0.17)	(0.50)	(0.17)	(0.29)	(3.45)	(0.04)	(0.13)	(1.05)	(5.00)	(0.07)	(2.22)	(0.05)	(0.63)	(5.64)	(0.37)
DTPA (soil) mg/kg	1.79	14.20	1.73	1.21	7.84	1.40	1.61	7.60	1.04	1.05	12.29	0.90	3.63	15.26	2.88
	(0.05)	(0.82)	(0.02)	(0.05)	(0.19)	(0.06)	(0.21)	(1.31)	(0.08)	(0.05)	(0.46)	(0.01)	(0.30)	(0.33)	(0.36)
NH_4NO_3 (soil) mg/kg	0.06	0.82	-0.03	0.02	4.69	0.01	0.02	7.38	0.63	0.04	1.91	-0.04	0.08	1.80	-0.02
	(0.01)	(0.05)	(0.00)	(0.00)	(0.24)	(0.00)	(0.00)	(0.84)	(0.26)	(0.00)	(0.09)	(0.00)	(0.00)	(0.07)	(0.00)
DGT µg/kg	0.84	123.70	0.15	1.61	296.00	0.67	1.63	136.30	4.60	0.95	326.40	0.39	1.18	24.90	0.46
	(0.05)	(0.85)	(0.04)	(0.15)	(22.58)	(0.03)	(0.12)	(4.75)	(0.03)	(0.03)	(23.09)	(0.23)	(0.04)	(13.78)	(0.22)

Appendix Table 3. Year 2 (2010/11) Mean micronutrient concentrations in soil and leaf material measured pre-experimental treatment applications by total acid digestion or using different soil extraction methods. The standard error of the mean is given in parentheses. Values in red are below or very close to the criteria used in section 3.2.3 for deciding whether soils are likely to respond to micronutrient additions.

Method	Site														
	Rothamsted (calcareous soil)			Woburn (light soil)			Bardney (organic soil)			Prickwillow (organic soil)			Stretham (organic soil)		
	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn
Total (soil) mg/kg	21.76 (1.31)	1495.00 (100.00)	88.77 (4.95)	8.16 (0.17)	387.50 (12.12)	44.01 (0.96)	28.66 (0.44)	126.00 (2.68)	51.64 (1.82)	28.58 (2.64)	578.10 (19.63)	56.51 (2.20)	24.17 (0.96)	241.80 (11.50)	58.95 (1.39)
EDTA (soil) mg/kg	1.17 (0.17)	9.03 (0.67)	1.77 (0.18)	2.43 (0.06)	40.99 (1.91)	2.70 (0.11)	2.65 (0.10)	23.85 (6.80)	4.52 (0.53)	5.49 (0.90)	29.96 (4.18)	5.53 (0.46)	3.84 (0.16)	13.61 (3.14)	4.60 (0.24)
DTPA (soil) mg/kg	1.58 (0.06)	5.71 (0.50)	1.59 (0.06)	1.21 (0.05)	7.84 (0.19)	1.40 (0.06)	0.05 (0.01)	0.89 (0.26)	0.36 (0.03)	2.01 (0.23)	4.57 (0.17)	3.52 (0.27)	0.62 (0.16)	1.20 (0.62)	1.68 (0.43)
NH_4NO_3 (soil) mg/kg	0.04 (0.01)	0.06 (0.01)	0.00 (0.00)	0.01 (0.00)	0.90 (0.03)	0.02 (0.00)	0.01 (0.00)	3.81 (1.12)	0.04 (0.01)	0.03 (0.00)	0.04 (0.01)	0.00 (0.00)	0.02 (0.00)	0.45 (0.08)	0.04 (0.01)
DGT µg/kg	0.74 (0.12)	800.80 (13.79)	0.27 (0.09)	1.53 (0.01)	838.80 (42.76)	0.86 (0.29)	0.89 (0.04)	75.09 (7.38)	2.19 (0.24)	1.47 (0.11)	75.11 (30.13)	0.84 (0.05)	1.17 (0.18)	102.80 (20.57)	1.87 (0.24)

Appendix Table 4. Year 3 (2011/12) Mean micronutrient concentrations in soil and leaf material measured pre-experimental treatment applications by total acid digestion or using different soil extraction methods. The standard error of the mean is given in parentheses. Values in red are below or very close to the criteria used in section 3.2.3 for deciding whether soils are likely to respond to micronutrient additions.

Method	Site														
	Rothamsted (calcareous soil)			Woburn (light soil)			Caythorpe (light soil)			Prickwillow (organic soil)			Waterbeach (organic soil)		
	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn
Total (soil) mg/kg	19.91 (0.35)	1319.0 (32.1)	103.60 (0.76)	11.29 (0.34)	199.40 (5.85)	75.33 (0.55)	11.53 (0.38)	511.0 (7.1)	81.13 (2.25)	32.54 (1.11)	619.0 (19.6)	85.04 (0.89)	24.24 (1.31)	313.80 (34.68)	55.77 (3.84)
EDTA (soil) mg/kg	1.00 (0.03)	15.45 (0.40)	1.72 (0.03)	3.54 (0.16)	9.35 (1.58)	5.94 (0.19)	1.75 (0.04)	64.86 (4.45)	2.36 (0.07)	5.47 (0.17)	24.89 (2.54)	8.28 (0.46)	0.79 (0.26)	3.85 (0.60)	2.92 (0.75)
DTPA (soil) mg/kg	1.14 (0.03)	7.72 (0.40)	1.33 (0.02)	1.55 (0.11)	1.31 (0.05)	3.34 (0.22)	0.52 (0.01)	6.92 (0.59)	1.00 (0.02)	1.79 (0.06)	4.12 (0.09)	4.77 (0.39)	0.51 (0.03)	1.90 (0.23)	1.52 (0.05)
NH_4NO_3 (soil) mg/kg	0.00 (0.00)	0.02 (0.00)	0.01 (0.00)	0.02 (0.00)	0.06 (0.00)	0.01 (0.00)	0.01 (0.00)	0.10 (0.06)	0.01 (0.00)	0.00 (0.00)	0.04 (0.01)	0.02 (0.00)	0.01 (0.00)	0.03 (0.00)	0.01 (0.00)
DGT µg/kg	0.82 (0.02)	22.95 (4.91)	0.26 (0.10)	3.25 (0.34)	32.10 (17.57)	2.70 (0.40)	0.68 (0.05)	201.20 (31.78	0.24 (0.09)	1.51 (0.13)	23.67 (2.12)	1.29 (0.09)	0.28 (0.02)	80.61 (23.50)	0.14 (0.06)

Appendix Table 5. Average monthly temperatures and rainfall for Rothamsted and Woburn 2010 (Departure from 30-year means (1971 - 2000) in brackets).

	Rothamsted 2010				Woburn 2010			
	Average temp °C		Rainfall mm		Average temp °C		Rainfall mm	
January	1.18	(-2.42)	47.3	(-22.32)	1.2	(-2.66)	44.8	(-10.68)
February	2.85	(-0.87)	77.2	(+28.44)	2.9	(-1.02)	79.6	(+38.80)
March	6.18	(+0.26)	45.2	(-8.72)	6.4	(+0.29)	35.2	(-14.37)
April	8.94	(+1.16)	18.7	(-34.83)	9.1	(+1.29)	19.8	(-32.80)
May	10.54	(-0.50)	38.4	(-11.33)	10.9	(-0.19)	55.8	(+3.07)
June	15.56	(+1.65)	23.5	(-36.71)	15.4	(+1.41)	38.0	(-20.79)
July	17.99	(+1.60)	31.6	(-10.41)	18.2	(+1.78)	16.8	(-28.84)
August	15.77	(-0.62)	127.6	(+73.87)	16.1	(-0.27)	151.8	(+97.30)
September	13.82	(+0.10)	59.7	(-1.31)	14.0	(+0.18)	51.0	(-7.44)
October	10.47	(+0.28)	84.9	(+10.25)	10.8	(+0.52)	46.4	(-18.27)
November	5.13	(-1.22)	54.8	(-11.40)	5.0	(-1.56)	34.0	(-23.37)
December	-0.33	(-4.88)	35.3	(-34.80)	-0.7	(-5.45)	18.0	(-41.67)
Year	9.01	(-0.45)	644.2	(-59.4)	10.0	(-0.02)	573.2	(-17.37)

Appendix Table 6. Average monthly temperatures and rainfall for Rothamsted and Woburn 2011 (Departure from 30-year means (1981 - 2010) in brackets).

	Rothamsted 2011				Woburn 2011			
	Average temp °C		Rainfall mm		Average temp °C		Rainfall mm	
January	3.8	(-0.14)	84.6	(+14.63)	3.8	(-0.32)	75.6	(+21.1)
February	6.2	(+2.30)	56.8	(+6.66)	6.4	(+2.28)	54.2	(+12.0)
March	6.5	(+0.27)	10.0	(-40.80)	6.7	(+0.23)	5.2	(-40.7)
April	12.2	(+3.82)	5.2	(-49.86)	11.8	(+3.36)	5.2	(-47.0)
May	12.5	(+1.02)	23.6	(-31.09)	12.6	(+1.11)	37.4	(-15.9)
June	14.3	(-0.18)	83.0	(+29.74)	14.4	(-0.09)	57.2	(+7.1)
July	15.4	(-1.42)	44.6	(-5.27)	15.2	(-1.68)	51.6	(+1.7)
August	15.8	(-0.90)	81.2	(+17.47)	16.0	(-0.72)	66.4	(+8.6)
September	15.3	(+1.21)	38.4	(-19.22)	15.5	(+1.37)	50.8	(-6.3)
October	12.8	(+2.23)	25.2	(-56.47)	12.9	(+2.22)	21.2	(-49.6)
November	9.3	(+2.55)	36.4	(-40.19)	9.4	(+2.53)	30.8	(-31.7)
December	5.7	(+1.43)	82.4	(+12.88)	5.8	(+1.47)	69.6	(+13.8)
Year	10.8	(+1.02)	571.5	(-161.5)	10.9	(+0.98)	525.2	(-126.8)

Appendix Table 7. Average monthly temperatures and rainfall for Rothamsted and Woburn 2012 (Departure from 30-year means (1981 - 2010) in brackets).

	Rothamsted 2012				Woburn 2012			
	Average temp °C		Rainfall mm		Average temp °C		Rainfall mm	
January	5.5	(+1.51)	58.0	(-11.97)	5.5	(+1.39)	35.0	(-19.53)
February	3.3	(-0.67)	24.7	(-25.44)	3.2	(-0.90)	18.6	(-23.56)
March	7.9	(+1.67)	34.7	(-16.13)	7.7	(+1.20)	27.0	(-18.91)
April	7.4	(-0.90)	168.6	(+113.57)	7.7	(-0.74)	132.0	(+79.79)
May	12.0	(+0.53)	52.6	(-2.08)	12.0	(+0.46)	68.8	(+15.54)
June	13.8	(-0.62)	166.5	(+113.21)	14.0	(-0.49)	149.0	(+98.93)
July	15.7	(-1.10)	128.4	(+78.57)	16.1	(-0.76)	76.0	(+26.11)
August	17.2	(+0.54)	54.9	(-8.82)	17.5	(+0.74)	56.6	(-1.20)
September	13.3	(-0.76)	40.4	(-17.27)	13.6	(-0.55)	41.2	(-15.91)
October	9.7	(-0.92)	115.8	(+34.13)	9.8	(-0.89)	112.0	(+41.17)
November	6.5	(-0.28)	100.4	(+23.79)	6.6	(-0.25)	90.2	(+27.73)
December	4.5	(+0.20)	114.2	(+44.67)	4.8	(+0.44)	98.8	(+43.05)
Year	9.7	(-0.07)	1059.2	(+326.23)	12.2	(+0.22)	905.2	(+253.20)