Contrasting effects of dwarfing alleles and nitrogen availability on mineral concentrations in wheat grain

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1 **Contrasting effects of dwarfing alleles and nitrogen availability on mineral**

2 **concentrations in wheat grain**

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28   **Abstract**

29   *Background and aim*   Concentrations of essential minerals in plant foods may have declined in modern

30   high-yielding cultivars grown with large applications of nitrogen fertilizer (N). We investigated the effect

31   of dwarfing alleles and N rate on mineral concentrations in wheat.

32   *Methods*   Gibberellin (GA)-insensitive reduced height (*Rht*) alleles were compared in near isogenic

33   wheat lines.   Two field experiments comprised factorial combinations of wheat variety backgrounds,

34   alleles at the *Rht-B1* locus (*rht-B1a*, *Rht-B1b*, *Rht-B1c*), and different N rates. A glasshouse experiment

35   also included *Rht-D1b* and *Rht-B1b+D1b* in one background.

36   *Results*   In the field, depending on season, *Rht-B1b* increased crop biomass, dry matter (DM) harvest

37   index, grain yield, and the economically-optimal N rate (N_{opt}). *Rht-B1b* did not increase uptake of Cu, Fe,

38   Mg or Zn so these minerals were diluted in grain. Nitrogen increased DM yield and mineral uptake so

39   grain concentrations were increased (Fe in both seasons; Cu, Mg and Zn in one season). *Rht-B1b* reduced

40   mineral concentrations at N_{opt} in the most N responsive season. In the glasshouse experiment, grain yield

41   was reduced, and mineral concentrations increased, with *Rht* allele addition.

42   *Conclusion*   Effects of *Rht* alleles on Fe, Zn, Cu and Mg concentrations in wheat grain are mostly due to

43   their effects on DM, rather than of GA-insensitivity on N_{opt} or mineral uptake. Increased N requirement in

44   semi-dwarf varieties partly offsets this dilution effect.

45

46

Introduction

Deficiencies of the micronutrients iron (Fe) and zinc (Zn) affect more than 2 billion people worldwide (WHO 2002). Other minerals such as magnesium (Mg) and copper (Cu) may also be inadequate in the diet of some people (White and Broadley 2009). Cereals are an important source of minerals in the human diet; providing 44% of the daily intake of Fe, 27% of Mg, 25% of Zn and 31% of Cu in the UK adult population (Henderson et al. 2003). There are concerns that the concentrations of essential minerals in plant foods may have declined as a result of increased yield (Davis et al. 2004; Garvin et al. 2006; White and Broadley 2005). Analysis of the archived wheat grain samples from the Broadbalk long-term experiment at Rothamsted has shown that the concentrations of Fe, Zn, Cu and Mg have all declined since the 1960s, coinciding with the introduction of modern short-straw cultivars (Fan et al. 2008).

Rapid increases in wheat yields in major wheat growing areas of the world from the 1960s through to the 1990's were associated with a reduction in wheat stature. This shortening was accompanied by an increase in dry matter harvest index, and also permitted sufficient nitrogen (N) fertilizer to be applied to the crop without the crop lodging such that canopy size could be optimized for capture of photosynthetically active radiation, and thus biomass yield (Gooding 2009). In the UK during the 1970s and 1980s yield increases were concurrent with final crop heights declining to around an apparently optimal stature of between 800 and 900 mm (Flintham et al. 1997; Austin 1999). Reductions in height were achieved either by the combined effect of many minor genes, and/or by the inclusion of major genes for reduced height (*Rht*). The commonly used *Rht-B1b* and *Rht-D1b* (previously named *Rht1* and *Rht2*) semi-dwarfing alleles from the Japanese wheat 'Norin 10' reduce sensitivity to endogenous gibberellins (GA) (Murase et al. 2008), and individually often reduce height by about 15% (Addisu et al. 2010). In the UK, *Rht-D1b* has been widely used in elite lines since the 1970's (e.g. cvs. Hobbit (1977) and Mardler (1978) were early examples (Austin et al. 1980)), although *Rht-B1b* has also been used more recently (e.g. cv. Robigus). More potent alleles, conferring severe dwarfism (>30 % height reduction) are available e.g. *Rht-B1c* (previously *Rht3*) from 'Tom Thumb' (Borner et al. 1997). The last widely used winter wheat cultivar suited for bread-making in the UK which did not have a major semi-dwarfing gene was cv. Mercia (introduced in 1983). Despite the lack of an *Rht* allele, the height of Mercia can be considered to be within the range commonly observed for modern commercial releases in the UK, that have an *Rht* allele (Gooding et al. 2012).

There is little information as to whether the reductions seen in mineral concentrations in UK wheat are due to effects of gibberellin insensitivity conferred by the adoption of the Norin 10 semi-dwarfing alleles, or due to yield increases associated with shortening *per se*. Here we use near isogenic lines varying for GA sensitivity conferred by alleles at the *Rht-B1* locus (*rht-B1a* (tall); *Rht-B1b* (semi-dwarf);

80 *Rht-B1c* (severe dwarf) in backgrounds that vary for height (Mercia, Maris Widgeon (1964) and Maris

81 Huntsman (1969)) in three different environments: field plots in two contrasting seasons, and pots under

82 glass (the Maris Huntsman series only).

83   Adoption of semi-dwarfing alleles increases the amount of N fertilizer that can be justified to be

84 applied by farmers on economic grounds (Loddo and Gooding 2012). Nitrogen availability has a

85 significant impact on mineral uptake and partitioning to, and concentrations in, the grain (Cakmak et al.

86 2010; Kutman et al. 2011a, b). To more fully evaluate the impact of the adoption of semi-dwarf cultivars

87 on the mineral concentrations on UK wheat it is, therefore, necessary to investigate any interaction

88 between dwarfing alleles and N fertilizer application rate, and more specifically, to evaluate mineral

89 concentrations at the economically optimal N rate for individual lines.

90   The aim of the present study was to quantify the effects of dwarfing alleles and the application rate

91 of N on the uptake of minerals and their concentrations in grain, especially Fe, Zn, Cu and Mg which are

92 often inadequate in the diet.

93

94 **Materials and methods**

95

96 Field experiments

97

98 Plots of winter wheat were maintained on a free-draining sandy loam soil (9% clay, particle size<0.2 μm;

99 19% silt, 2-20 μm; 72% sand, 20-2000 μm) overlying coarse red-brown sand, principally of the Sonning

100 series (Jarvis, 1968), at the Crop Research Unit, University of Reading, UK (51°29'N, 0°56'W, 35m

101 above sea level), in the 2009/10 and 2010/11 growing seasons. In both seasons, the experiments followed

102 a three year rye grass and chicory ley, destroyed with glyphosate before mouldboard ploughing to 300

103 mm (Table 1). Before cultivation, the soil was tested for pH, phosphorous, potassium and magnesium

104 availability (Anon. 1986; Anon. 2010; Table 1) and corrective nutrients applied accordingly. Following

105 cultivation in autumn, total soil carbon and mineral N content (0 - 900 mm) averaged 0.69% DM and 55

106 kg N/ha respectively. Soil mineral N content in the following February averaged 35 Kg N/ha. For this

107 rotational position at this site over five previous seasons the N uptake by the above ground wheat

108 receiving full crop protection but no N fertilizer averaged 75 kg N/ha, i.e. a soil N supply index of 1

109 (Anon. 2010). Crop husbandry was indicative of local commercial practice (Table 1), and achieved a high

110 level of control of weeds, pests and diseases. Rainfall and air temperature were recorded at an automatic

111 weather station within 400 m of the experiments (Table 1).

112

113   Near isogenic lines (NILs) comprised a factorial combination of background varieties (Maris

114   Huntsman, Maris Widgeon and Mercia in 2009/10 and just Maris Widgeon and Mercia in 2010/11) and

115   three alleles at the *Rht-B1* locus: *rht-B1a* (tall), *Rht-B1b* (semi-dwarf, syn. *Rht1*) and *Rht-B1c* (severe-

116   dwarf, syn. *Rht3*). Untreated seeds ($300/m^2$) were drilled into 120 mm rows in 2 x 6 m sub-plots,

117   separated by a 500 mm double track wheeling. Main plots, randomized in three complete blocks,

118   comprised the background x allele combinations; each contained randomized sub-plots allocated to

119   receive different rates of N fertilizer applied as granular ammonium nitrate. In 2009/10 the total N rates

120   were 0, 100, 200 and 350 kg N/ha. For the three sub-plots receiving N, 50kg N/ha was applied at the first

121   node detectable growth stage (GS 31, Zadoks et al. 1974) and again at flag leaf emergence (GS 39). The

122   200 and 350 kg N/ha treatments received a further 100 and 250 kg N/ha respectively at the second node

123   stage (GS 32). In 2010/11 the total N rates were 0, 50, 100, 200, 350 and 450 kg N/ha applied in equal

124   splits at GS 31 and 33.

125   Crop height was measured five days before harvest with a polystyrene rising disc (Addisu et al.

126   2009). During the week before harvest, above ground whole crop rows either side of three randomly

127   placed 0.5 m lengths (total area equivalent of 0.36 m^2) were collected from each subplot. Samples were

128   partitioned into grain, chaff and straw. After oven drying at 80^oC, dry weight of each component was

129   estimated and mineral content assessed. Plot combine harvesting was with a 1.4 m cutter bar such that

130   effective separation of subplots was 0.8 m to reduce edge effects. Information from sample areas was

131   used to calculate harvest indices of dry matter and minerals.

132

133   Pot experiment

134

135   Soil (0 – 200 mm) was collected from the Rothamsted farm, air dried and sieved through a 5 mm sieve.

136   Plastic pots (200 mm diameter) were filled with 3.5 kg air-dried soil each. Fertilizers (0.7 g N as NH_4NO_3

137   and 0.175 g P as KH_2PO_4) were mixed into the soil. Seeds of the NILs of different *Rht* alleles (*rht-*

138   *B1a/D1a, Rht-B1b, Rht-D1b, Rht-B1b+D1b, Rht-B1c*) in the Maris Huntsman background were

139   germinated on perlite and vernalized at 5^oC in a controlled environment room for 8 weeks. Seedlings

140   were then transplanted into the soil (5 per pot). Each NIL was planted in four pots. Pots were placed on

141   benches in a randomized block design inside a glasshouse lit with sunlight supplemented with sodium

142   vapor lamps to maintain a light intensity of >350 µmol/m^2/s. Plants were watered daily with deionized

143   water to approximately 70% of the water holding capacity. At maturity, plants were harvested by cutting

144   at 1 cm above the soil surface. Grain was separated from the straw and dried at 80^oC for 48 h.

145

146

147 Chemical analysis

148

149 Grain and straw were ground to fine powder and digested with $HNO_3/HClO_4$ (85/15, v/v) in a heating

150 block (Zhao et al. 1994). The concentrations of minerals, including the major nutrients K, P, S, Ca and

151 Mg, the micronutrients Fe, Zn, Cu and Mn, and the soil contamination indicator mineral Ti, were

152 determined using inductively-coupled plasma atomic emission spectrometry. A certified reference

153 material (NIST 1567a wheat flour) was included in the analysis for quality assurance. The results

154 obtained were in good agreement with the certified values; mean and standard deviation from 18

155 digestion repeats were 14.6 ± 1.5 mg/kg for Fe (certified value 14.1 ± 0.5), 2.3 ± 0.2 mg/kg for Cu

156 (certified value 2.1 ± 0.2), 12.6 ± 0.8 mg/kg for Zn (certified value 11.6 ± 0.4) and 386 ± 9 mg/kg for Mg

157 (certified value 400 ± 2). Nitrogen concentration was determined with the Dumas combustion method

158 (LECO FP-328, LECO, Stockport, UK).

159

160 Statistical analysis

161

162 For the field experiments, appropriate split plot analyses of variance (ANOVA) included a treatment

163 structure of Background*Allele*pol(N rate;3) (GENSTAT 13; VSN International, Hemel Hempstead,

164 UK) so as to split N effects into polynomial contrasts. Titanium (Ti) on the above-ground crop

165 (straw+chaff+grain) was used as a covariate for above ground yields and harvest indices of DM and

166 minerals to help correct for error associated with any soil contamination. The effect of the covariate was

167 significant ($P<0.05$) in all these cases. Ti has been used as an indicator of soil contamination because it is

168 abundant in soil but hardly taken up by plants (Cook et al. 2009). There were no significant effects of the

169 covariate on any assessments of the grain (yields, mass per grain, and concentration) and therefore, the

170 covariate was omitted from grain ANOVAs. There was little evidence of Background x Allele x N rate

171 interactions so data are presented for the two factor interactions including N rate. The economically

172 optimal level of N fertilizer was calculated on the simple basis of the N rate fitted to give the greatest

173 financial margin over N cost. These calculations were based on N:grain price ratios of 5:1 and 10:1

174 (Sylvester-Bradley and Kindred 2009) with grain traded on a 15% moisture content basis. Grouped

175 polynomial regression analysis was employed to assess the relationships between mineral and N

176 concentrations. For the pot experiment, ANOVA was performed to test the significance of the differences

177 between the NILs.

178

179

180 **Results**

181

183

The 2009/10 growing season was characterized by comparatively dry conditions during stem extension (April and May; Table 1) and grain filling (June and July).

Averaged over background and N rate, the inclusion of the Norin 10 semi-dwarfing *Rht-B1b* allele produced crop heights in the 800-900 mm range i.e. that predicted to be optimal for yield (Table 2). The *rht-B1a* and *Rht-B1c* alleles were associated with crop heights which would be considered to be supra- and sub- optimal respectively. *Rht-B1b* increased grain yield of DM (Fig. 1*c*) because it increased both above-ground crop biomass (Fig. 1*a*), and harvest index (Fig. 1*b*). Relative to *rht-B1a* (tall), *Rht-B1b* was associated with significantly ($P<0.05$) reduced concentrations of Cu in grain (Fig. 1*i*; Main Effect = -2.6 mg/kg; s.e.d. = 0.0768 (d.f.=16)), Fe (Fig. 1*n*; -2.7; 0.71), Mg (Fig. 1*s*; -98.4; 12.77), and Zn (Fig. 1*x*; -2.6; 0.50). *Rht-B1b* also significantly reduced the concentrations of N (Fig. 2), manganese (Mn), phosphorous (P), and sulfur (S), but not of calcium (Ca), potassium (K), molybdenum (Mo) or sodium (Na) (data not presented). The decreased concentrations of Cu, Fe, Mg and Zn can only be ascribed to dilution due to increased yields of dry matter, as there was no significant effect of *Rht-B1b* on total mineral uptake, whether expressed in terms of the above ground biomass or grain per unit area, or on a per grain basis (Fig. 1).

Rht-B1c improved harvest index relative to *Rht-B1b* (Fig. 1*b*) but this was insufficient to counter a negative effect on above ground biomass (Fig. 1*a*), such that severe dwarfing was associated with reduced grain yields (Fig. 1*c*). *Rht-B1c* was not, however, significantly (P>0.05) detrimental to the accumulation of Cu (Fig. 1*e,g*), Fe (Fig. 1*j,l*), or Zn (Fig. 1*t,v*) so relative to *Rht-B1b*, the grain concentrations of all three mineral were increased (Fig. 1*i,n*). For Mg, as for other minerals, *Rht-B1c* did not influence uptake into the above-ground crop (Fig. 1*o*). In contrast to effects on DM, however, severe dwarfing was associated with a reduced harvest index of Mg (Fig. 1*p*) such that the yields of Mg per grain were reduced by *Rht-B1c* (Fig. 1*q*), contributing to reduced concentrations of Mg in the grain (Fig. 1*s*).

In the absence of *Rht*, the height of Mercia would be considered optimal and Maris Widgeon supra-optimal (Table 2). The two shorter cultivars had the highest DM harvest indices and apparently, also the higher grain yields (P=0.044 for the main effect of background). Similar to the results for *Rht-B1b*, there was no significant effect of background on mineral uptake (Cu, Fe, Mg or Zn) into the above-ground biomass, and mineral concentrations tended to be more dilute in Mercia compared with Maris Widgeon (Table 2, $P<0.001$, 0.077, <0.001 for the main effects of background on Cu, Mg and Zn respectively). Additional effects of cultivar were, however, evident: the harvest indices and grain yields per unit area for

214 all four minerals varied significantly among the backgrounds, and were always highest for Maris
215 Huntsman (Table 2).

216   In contrast to the effects of *Rht-B1b* which diluted mineral concentrations as grain yields of dry
217 matter were increased, N rate was associated with both increased grain DM yields, *and* increased
218 concentrations of Cu and Fe. The quantity of Fe per grain was also increased by N applications (Fig. 1*h*,
219 *m*, *r*, *w*) even though such applications also reduced mean grain weight (Fig. 1*d*). Nitrogen application
220 increased the quantities of all four minerals in the above ground crop biomass (Fig. 1*e*, *j*, *o*, *t*), which
221 largely explains similar effects on quantities in the grain on a unit area basis (Fig. 1*g*, *l*, *q*, *v*), although N
222 application also increased the harvest index of Fe (Fig. 1*k*). There was a strong association between the
223 grain Fe concentration and that of grain N (Fig. 2); to the extent that when grain N concentration was
224 included in the regression model, there was no statistical justification for including effects of Allele as an
225 explanatory factor. This was not the case for Cu, Mg and Zn (Fig. 2).

226   The economically optimal level (Table 3) of N fertilizer varied with allele because there was a
227 significant Allele x N rate interaction on grain yield of DM (Fig. 1*c*). The grain yield of *Rht-B1b* was the
228 most responsive to N (Fig. 1*c*), partly because of a combination of comparatively high biomass yield (Fig.
229 1*a*) and harvest index (Fig. 1*b*) at the highest N rate. Using the quadratic fits in Fig. 1*c*, N_{opt} was between
230 40 and 50 kg N/ha higher for *Rht-B1b* compared with *rht-B1a* (Table 3). Despite the benefit of N
231 application for some mineral concentrations, however, this level of increase in N application was
232 insufficient to compensate for the diluting effect of *Rht-B1b* (Table 3) i.e. semi-dwarfing reduced the
233 concentrations of all four minerals whether compared at a single average N rate, or at the different N_{opt} for
234 the different alleles. Increasing N cost relative to grain value reduced N_{opt} and therefore tended to reduce
235 Fe concentration at N_{opt}.

236
237 Field experiment 2010/11
238

239 Conditions were particularly dry during the start of stem extension (March and April, Table 1), and also
240 exceptionally warm during April. The period of below average rainfall continued in May. The grain
241 filling period (June and July) was associated with above average rainfall and comparatively cool
242 temperatures. The notably dry conditions during spring, coinciding with the timing of the N application,
243 probably contributed to plants being shorter than in the previous season (Tables 2 and 3), and also to the
244 crop being much less N responsive in terms of above ground biomass and grain yield (Fig. 3*a,c*). In
245 contrast to the previous year, crop heights of *rht-B1a* would not be considered excessive for optimizing
246 grain yields (Table 2). Also in contrast to 2009/10, there were no significant main effects of *Rht-B1b* on
247 above-ground dry matter or grain yield despite a small positive effect on harvest index (Fig. 3*a,b,c*).

248   Relative to *rht-B1a* (tall), the size of effects of *Rht-B1b* on grain mineral concentrations were much

249   reduced compared with the previous year. Indeed, for the 2011 harvest there was no statistically

250   significant main effect of *Rht-B1b* on the grain concentrations of Cu, Fe, Zn (Fig. 3*i*, *n*, *x*), Ca, Mn, Na or

251   S (data not presented). *Rht-Blb* was associated with small but significant reductions in the concentrations

252   of Mg (Fig. 1*s*; Main Effect = -31 mg/kg; s.e.d. = 9.6 (d.f.=16)), N (Fig. 2; -0.84; 0.366) and P (-110; 35),

253   and an increase in the concentration of K (+139; 34.1). Where effects were statistically significant, it is

254   difficult to ascribe these directly to effects on dilution with DM, mineral uptake, or partitioning given the

255   very marginal effects of *Rht-B1b* on any of these variables.

256   As in 2010, *Rht-B1c* improved harvest index relative to *Rht-B1b* (Fig. 3*b*) but in 2011 this was

257   sufficient to counter the negative effect on above ground biomass (Fig. 3*a*), such that severe dwarfing was

258   associated with comparable grain yields to taller lines, when taking the averages over N rates (Fig. 3*c*). As

259   well as above ground dry matter accumulation *Rht-B1c* was detrimental to the accumulation of Mg (Fig.

260   3*o*) and Zn (Fig. 3*t*), particularly at high rates of N fertilizer. In contrast to the situation with DM, *Rht-B1c*

261   did not increase the harvest index of minerals, and this failure contributed to the reduction in

262   concentration of Mg in the grain (Fig. 3*s*).

263   In 2011, grain yields of Mercia tended to be higher than Maris Widgeon, associated with

264   significantly reduced crop height and increased DM harvest index (Table 3). Harvest index of Cu was

265   increased in Mercia, but this was insufficient to prevent a dilution of Cu in the grain. Zn was also more

266   dilute in Mercia grain, again not because of reduced uptake or partitioning, but because effects did not

267   match those on DM.

268   As in 2010, uptake of Cu, Fe, Mg and Zn increased with N rate, both in the above ground biomass

269   and in the grain (Fig. 3). Increases in mineral quantity in the grain tended to be greater than the grain DM

270   yield response to N application such that, for at least part of the range of rates used, mineral concentration

271   increased with N application: for Cu to about 100 kg N/ha (Fig. 3*i*); for Fe and Mg, to about 300 kg N/ha

272   (Fig. 3*n*, *s*); and for Zn to about 400 kg N/ha (Fig. 3*x*). The concentration of Fe in the grain was again

273   closely associated with N concentration (Fig. 2). There were also positive relationships between grain N

274   concentration and the concentrations of Mg and Zn, but not to the extent that N concentration could

275   account for *Rht* allele effects.

276   Although there was no main effect of *Rht* allele on grain DM yield, there was a significant Allele x N

277   rate interaction (Fig. 3*c*). *Rht-B1b* was again the most N-responsive allele, having a comparatively low

278   yield at low N rates, but the highest yield at high N rates. The derived N_{opt} for a 5:1 N:grain price ratio

279   suggested that it would have been justified to apply 64 kg more N/ha on *Rht-B1b* than on *rht-B1a* (Table

280   4). Given the shallow response of grain yield to N in this season, however, the statistical error around this

281   figure must be substantial. Nonetheless, if an extra 64 kg N was applied to *Rht-B1b* this would have been

282 sufficient to negate the diluting effect of the allele on the Mg concentration in the grain. It should also be

283 noted, however, that the grain yield response was so shallow, that there was no economic justification for

284 applying any N fertilizer to any allele if the N:grain price ratio was as high as 10:1 (Table 4), and hence

285 the diluting effect of *Rht-B1b* on Mg concentration would have persisted at high N cost.

286

287 Pot experiment

288

289 Both plant height and grain yield decreased with the introduction of dwarfing alleles in the Maris

290 Huntsman background in the order of *rht-B1a/D1a* (tall) > *Rht-B1b* > *Rht-D1b* > *Rht-B1B+D1b* > *RhtB1c*

291 (Fig. 4a). In contrast to the field experiments, *Rht-B1b* did not increase the grain yield compared with *rht-*

292 *B1a/D1a* (tall). Plant heights of all lines were substantially shorter than the corresponding ones in the

293 field experiments.

294   The concentrations of Fe, Zn and Cu differed significantly ($P < 0.001$) between the dwarfing alleles,

295 but, in contrast to the field experiments, they increased with the severity of the dwarfing phenotype (Fig.

296 4b). Note that the concentrations of Fe and Zn were considerably larger than those from the field trial. In

297 addition to the three minerals presented here, the concentrations of Mg, P and S also showed significant

298 increasing trends with the degree of dwarfing (data not shown). There were highly significant negative

299 correlations between the concentrations of Fe, Zn or Cu and grain yield (r = -0.80, -0.79 and -0.71 for Fe,

300 Zn and Cu, respectively, $P<0.001$; Fig. 5). The amounts of Fe, Zn and Cu taken up by the plants (straw

301 and grain) did not differ significantly between the different *Rht* alleles (Fig. 4c), nor were there any

302 significant differences in the amounts of these minerals contained in the grain (data not shown). The

303 harvest indices for both the biomass and the three minerals were broadly similar, showing a decreasing

304 trend with the dwarfing severity (Fig. 4d).

305

306 **Discussion**

307

308 We confirm the shortening effects of alleles at the *Rht-B1* and *Rht-D1* loci (Flintham et al. 1997). Also

309 consistent with previous studies, we find semi-dwarfing is beneficial for grain yield when it reduces final

310 crop height to around 800-900 mm in UK conditions (Flintham et al. 1997; Austin 1999), and that semi-

311 dwarfing increases the response to N fertilizer in such circumstances (Anderson et al. 1991). That *Rht-*

312 *B1c* reduces grain yield because increases in harvest index are insufficient to compensate for reductions in

313 above ground biomass was also reported by Flintham et al. (1997). The glasshouse pot experiment here

314 was consistent with other studies where semi-dwarfing with *Rht* alleles have had neutral or negative

effects on yield in situations where *rht-B1a/D1a* is associated with plant heights already optimal or sub-optimal for yield (Flintham et al. 1997; Chapman et al. 2007; Gooding et al. 2012).

The main objective of the present study was to test if the introduction of the GA-insensitive dwarfing alleles in wheat affects the concentrations of minerals in grain that are particularly important for human nutrition. Our results suggest that the dwarfing alleles have comparatively little direct effects on the uptake and translocation to grain of Fe, Zn, Cu and Mg; however, the concentrations of these minerals in grain were affected indirectly mainly as a result of the changes in grain yield. That the dilution of minerals by the incorporation *Rht-B1b* was not due to more direct physiological effects of gibberellin insensitivity is further supported by: a lack of progression of effects through *rht-B1a* to *Rht-B1b* to *Rht-B1c* on mineral concentration despite the progression in GA insensitivity and degree of dwarfing; the contrasting effects of *Rht* alleles in the two field experiments, and between the field and pot experiments, consistent with the contrasting effects on grain yield; and a similar dilution of minerals between Maris Widgeon and Mercia, associated with reduced height but not mediated through differences in GA-sensitivity.

Consistent with the effects of *Rht-B1b* and Mercia in the field, mineral (e.g. Zn, Fe or Mg) concentrations have been found to correlate negatively with grain yield among diverse wheat cultivars (McDonald et al. 2008; Morgounov et al. 2007; Oury et al. 2006; Zhao et al. 2009). The decreasing trend of mineral concentrations in wheat grain, either with the cultivar release date (Garvin et al. 2006; Zhao et al. 2009) or in the Broadbalk long-term experiment (Fan et al. 2008), can be largely attributed to the dilution effect brought about by increased grain yield. This effect may arise because the distribution of minerals to grain does not match the increased biomass allocation to grain in the higher yielding cultivars.

Whilst yield gain through cultivar improvement results in a dilution of mineral concentrations in wheat grain, yield increase in response to increasing the application rate of N did not. In fact, increasing N rate increased the concentrations of Fe and, depending on season and to a lesser extent, Cu, Mg and Zn in grain. Similar effects have been reported for Fe and Zn in durum (*Triticum durum*) and bread (McGrath 1985; Cakmak et al. 2010; Shi et al. 2010) wheats. Increasing N supply was found to increase the uptake, root-to-shoot translocation and remobilization from vegetative tissues to grain of Zn and Fe in durum wheat, possibly because of more transporter proteins and an increased pool of nitrogenous chelators (e.g. nicotianamine) that are important for the phloem transport of these metals (Kutman et al. 2011*a*, *b*). Our results relating grain mineral concentration to grain N concentration suggest that any effect of plant N compounds on Fe is much more direct than that for Cu or Zn. Our results would suggest that the benefits of N rate for Zn are more reliable as grain N concentrations exceed 20 g N/kg. Grain N concentrations were in a lower range in the 2009-10 than in the 2010-11 trial, which may explain the lack of a significant N effect on grain Zn in the former season.

349   We demonstrate that the greater yield responses of appropriately-statured cultivars justify the use of
350   larger N rates. This has the effect of offsetting some of the dilution effect on grain mineral concentration
351   but at the economically optimal level of N the concentrations of minerals were still about 10% lower for
352   the semi-dwarf *Rht-B1b* allele than in the tall allele in the most responsive field season of 2009/10 (Table
353   3). It is well known, however, that wheat responses to N rate, and its interaction with genotype varies with
354   site and season, so further experiments would be necessary to quantify this offset more generally. Our
355   field experiments were conducted in two comparatively dry seasons for the site, which would be expected
356   to reduce the wheat response to N (Gooding 2009). A comparison of the two seasons reported here
357   strongly supports the importance of adequate moisture for large responses of wheat grain yield to N rate.
358   The comparison also suggests that offsetting of the *Rht-B1b* mediated dilution of minerals with the extra
359   N fertilizer is less likely in wetter seasons. Future benefits of N for mineral concentrations (particularly
360   for Fe) are likely to be curtailed if increased cost and restriction of N fertilizer continues, and/or genetic
361   improvements in N utilization efficiency (crop DM / crop N; Gooding et al. 2012) are realized. There is,
362   therefore, increasing need to explore further the genetic variability in grain mineral concentration to
363   achieve both high yields and sufficient levels of minerals in wheat (White and Broadley 2009; Zhao et al.
364   2009).

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376   **References**

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Table 1 Monthly weather data, soil analyses, and crop husbandry common to all treatments for the two field plot experiments

	2010		2011		Long term average (47 years)	
	Rainfall (mm)	Mean temp. (°C)	Rainfall (mm)	Mean temp. (°C)	Rainfall (mm)	Mean temp. (°C)
March	46.2	6.3	13.8	6.5	47.2	6.5
April	22.0	9.2	2.8	12.2	47.9	8.7
May	12.0	11.2	30.0	12.3	47.9	12.0
June	20.8	16.1	89.8	14.2	49.3	15.0
July	31.6	18.4	41.1	16.2	44.6	17.2
August	108.0	16.1	125.2	15.9	60.1	17.0

Treatment or operation	Growth stage (GS, Zadoks et al. 1974)		
Soil test 0-90cm			
pH		6.3	6.4
P mg/l (index)		49.0 (4)	26.4 (3)
K mg/l (index)		99 (1)	88 (1)
Mg mg/l (ind.)		48 (1)	52 (2)
Herbicide	Destruction of previous sward	Glyphosate 1.8kg/ha	
Sub-soiled	Pre-ploughing	16 September	Not applicable
Nutrition	Pre-ploughing	120 kg/ha K_2O equ. as KCl	90 kg/ha K_2O equ. as KCl
Ploughed		8 October	23 September
Harrowed		21 October	8 October
Drilled		22 October	8 October
Rolled		22 October	11 October
Herbicide	13	1.2 kg/ha pendimethalin + 20 g/ha carfentrazone-ethyl + 10 g/ha flupyrsulfuron-methyl	
Nutrition	30	40 kg/ha SO_3 equ. as K_2SO_4	32 kg/ha SO_3 equ. as K_2SO_4
Herbicide	31	50 g/ha thifensulfuron-methyl + 5 g/ha metsulfuron-methyl	
Fungicide	32	125 g/ha epoxiconazole + 150 g/ha quinoxyfen + 1 kg/ha chlorothalonil	125 g/ha epoxiconazole + 150g/ha metrafenone + 1 kg/ha chlorothalonil
Fungicide	39	125 g/ha epoxiconazole + 250 g/ha azoxystrobin	
Fungicide	59	250 g/ha tebuconazole	
Insecticide	59	5 g/ha deltamethrin	
Harvest		9 August	17 August

Table 2 The effect of allele at the *Rht-B1* locus and nitrogen fertilizer application rate (N, kg/ha) on the final crop height (mm) of winter wheat.

2010 (Mean of Maris Huntsman, Maris Widgeon and Mercia backgrounds)				2011 (Mean of Maris Widgeon and Mercia backgrounds)			
N	*B1a*	*B1b*	*B1c*	N	*B1a*	*B1b*	*B1c*
0	910	720	420	0	840	700	450
100	1020	830	460	50	850	720	460
200	1020	840	480	100	830	700	440
350	1000	830	470	200	820	730	440
				350	830	750	460
				450	810	740	450
S.E.D. [a]			11				13
d.f.			54				55
Mean	990	810	460		830	720	450
S.E.D. [b]			12				19
d.f.			16				10

459   [a]for comparing N levels within allele; [b]for comparing allele means

460

Table 3 Effect of cultivar on the height, yields and partitioning of dry matter and minerals in winter wheat.

	2010				2011		
	Maris Hunts-man	Mercia	Maris Widg-eon	S.E.D. (16 d.f.)	Mercia	Maris Widg-eon	S.E.D. (10 d.f.)
Crop height (mm)	1010	820	1120	20	750	910	27
Dry Matter							
Above ground crop (t/ha)	13.9	13.0	15.0	1.23	9.9	8.8	1.50
Harvest_index	0.47	0.47	0.39	0.013	0.54	0.48	0.007
Grain yield (t/ha)	6.85	6.23	5.82	0.535	5.33	4.24	0.556
Mass per grain (mg)	49.5	40.7	48.9	1.28	54.8	59.1	0.74
Copper (Cu)							
Above ground crop (mg/m^2)	4.32	3.85	4.25	0.411	2.88	2.96	0.439
Harvest_index	0.70	0.63	0.60	0.021	0.66	0.62	0.011
Grain yield (mg/m^2)	3.10	2.41	2.54	0.201	1.91	1.88	0.199
Mass per grain (μg)	0.22	0.16	0.21	0.006	0.20	0.26	0.006
Grain concentration (mg/kg)	4.52	3.87	4.32	0.133	3.60	4.42	0.105
Iron (Fe)							
Above ground crop (mg/m^2)	53.4	51.8	57.6	7.02	184.1	184.9	9.90
Harvest_index	0.41	0.36	0.33	0.026	0.11	0.09	0.013
Grain yield (mg/m^2)	20.6	18.5	18.3	1.88	15.8	12.7	1.68
Mass per grain (μg)	1.42	1.16	1.48	0.068	1.61	1.75	0.057
Grain concentration (mg/kg)	28.8	28.7	30.1	1.23	29.2	29.5	0.99
Magnesium (Mg)							
Above ground crop (g/m^2)	1.13	1.03	1.15	0.119	0.96	0.80	0.164
Harvest_index	0.59	0.59	0.52	0.013	0.63	0.58	0.028
Grain yield (g/m^2)	0.68	0.61	0.59	0.046	0.60	0.46	0.062
Mass per grain (μg)	50.0	40.4	49.9	1.00	61.3	64.4	1.11
Grain concentration (g/kg)	1.01	0.99	1.02	0.022	1.12	1.09	0.014
Zinc (Zn)							
Above ground crop (mg/m^2)	15.6	14.6	16.1	1.63	12.4	11.6	1.52
Harvest_index	0.85	0.85	0.79	0.017	0.92	0.90	0.008
Grain yield (mg/m^2)	13.3	12.2	12.6	1.09	11.2	10.2	1.02
Mass per grain (μg)	0.97	0.80	1.07	0.035	1.15	1.42	0.063
Grain concentration (mg/kg)	19.6	19.8	21.9	0.86	20.9	24.0	1.16

461   Values are means over N fertilizer rates and three replicates.
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Table 4 The effect of allele at the *Rht-B1* locus on the fitted economically optimal level of nitrogen (N_{opt}) for two N:Grain price ratios, and the fitted (Figs. 1 & 3) mineral concentrations at N_{opt}.

N:Grain price ratio	2010			2011		
	B1a	*B1b*	*B1c*	*B1a*	*B1b*	*B1c*
N_{opt} (kg/ha)						
5.0:1	253	299	243	115	179	97
10.0:1	213	252	197	0	0	0
Grain yield at N_{opt}						
5.0:1	7.8	9.2	7.3	4.7	4.9	4.9
10.0:1	7.6	9.0	7.1	4.1	3.9	4.4
mg Cu/kg at N_{opt}						
5.0:1	4.3	4.0	4.9	4.2	4.3	4.2
10.0:1	4.3	4.0	4.8	3.8	3.9	4.1
mg Fe/kg at N_{opt}						
5.0:1	33.9	30.4	33.9	28.9	30.7	28.3
10.0:1	32.8	29.3	31.8	24.0	24.0	24.0
g Mg/kg at N_{opt}						
5.0:1	1.01	0.88	0.88	1.10	1.10	1.01
10.0:1	1.00	0.87	0.88	1.05	1.02	0.97
mg Zn/kg at N_{opt}						
5.0:1	19.6	17.4	20.4	21.5	22.7	21.2
10.0:1	19.5	16.9	19.8	18.8	18.8	18.8

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468 **Figures:**

469 **Fig. 1** Effect of nitrogen and allele at the *Rht-B1* locus on yield and mineral content of winter wheat near

470 isogenic lines. □=*rht-B1a* (tall); △ = *Rht-B1b* (semi-dwarf); ○, dashed line = *Rht-B1c* (severe dwarf).

471 Points are means of three replicate blocks and three wheat backgrounds (Maris Huntsman, Mercia, Maris

472 Widgeon). Left and right error bars are S.E.D. for comparing between and within alleles respectively (sub

473 plot error d.f. = 53). Main and interacting effects of Allele, Linear N and Quadratic N only fitted if effect

474 is statistically significant (*P*<0.05).

475 **Fig. 2** Relationships between grain mineral concentration and grain nitrogen concentration in wheat

476 harvested in 2010 (left) and 2011 (right). Points are means of three replicate blocks and wheat

477 backgrounds (Mercia, Maris Huntsman and Maris Widgeon in 2010; Mercia and Maris Widgeon in

478 2011), and vary for allele as near isogenic lines at the *Rht-B1* locus (□=*rht-B1a* (tall); △ = *Rht-B1b*

479 (semi-dwarf); ○, dashed line = *Rht-B1c* (severe dwarf)) and for nitrogen fertilizer application rate (N

480 rate). Numerals above inverted triangles in Fe panels are the N rates (kg/ha) associated with the nitrogen

481 concentrations achieved (averaged over allele). Error bars are maximum S.E.D. for comparing points (sub

482 plot error d.f. = 53 and 55 for 2010 and 2011 respectively). Main and interacting effects of Allele, Linear

483 N and Quadratic N only fitted if effect is statistically significant (*P*<0.05).

484 **Fig. 3** Effect of nitrogen and allele at the *Rht-B1* locus on yield and mineral content of winter wheat near

485 isogenic lines harvested in 2011. □=*rht-B1a* (tall); △ = *Rht-B1b* (semi-dwarf); ○, dashed line = *Rht-B1c*

486 (severe dwarf). Points are means of three replicate blocks and two wheat backgrounds (Mercia, Maris

487 Widgeon). Left and right error bars are S.E.D. for comparing between and within alleles respectively (sub

488 plot error d.f. = 55). Main and interacting effects of Allele, Linear N and Quadratic N only fitted if effect

489 is statistically significant (*P*<0.05).

490 **Fig. 4** Effects of dwarfing alleles in the Maris Huntsman background on grain yield and height (a), grain

491 micronutrient concentrations (b), uptake (c) and harvest indices in the pot experiment. Error bars are

492 S.E.D. (d.f. = 12) for comparing different alleles; the error bars from left to right in each graph correspond

493 to the variable from top to bottom in the legend box.

494 **Fig. 5** Correlation between grain Fe, Zn or Cu concentrations and grain yield in the pot experiment.
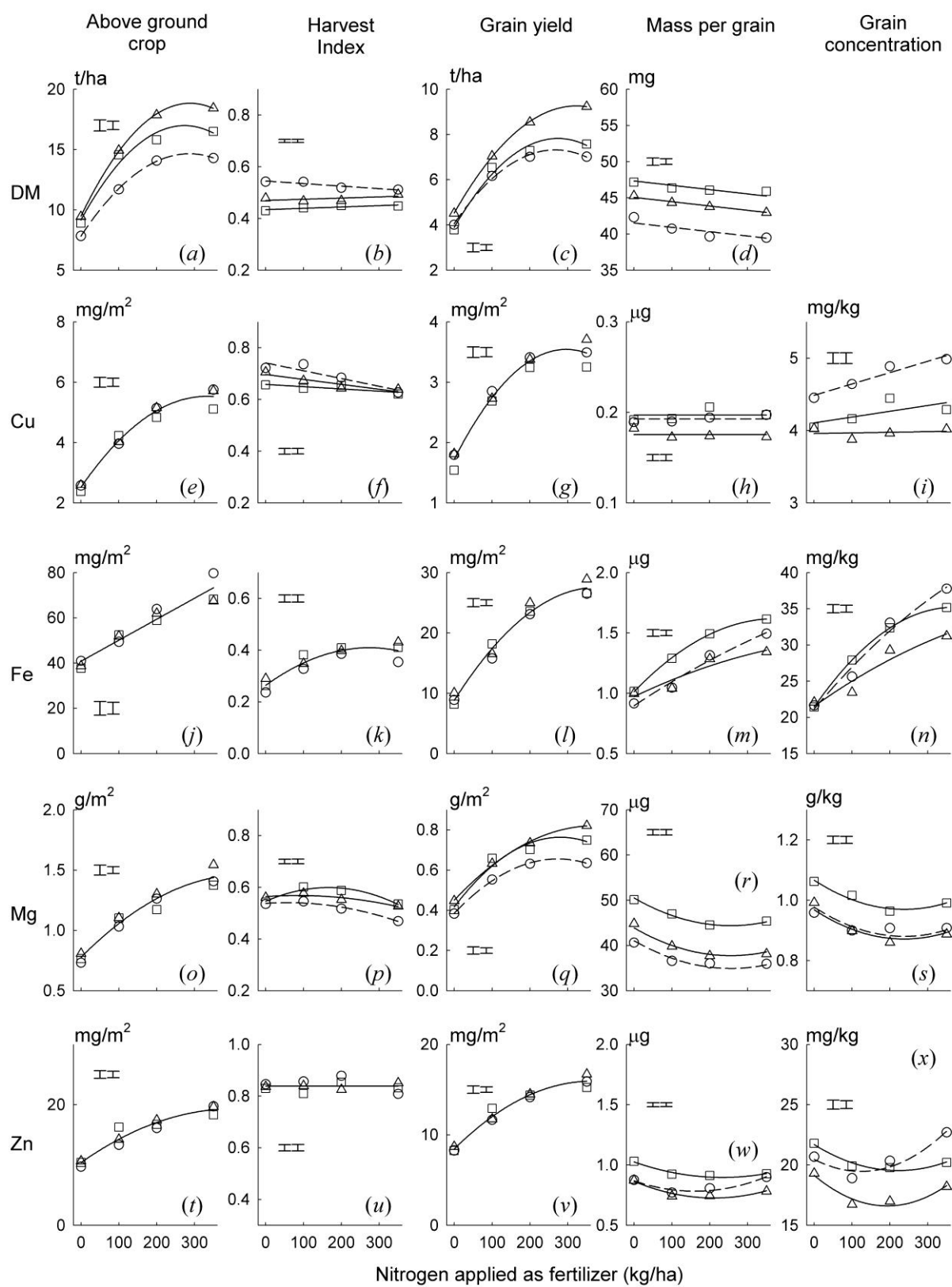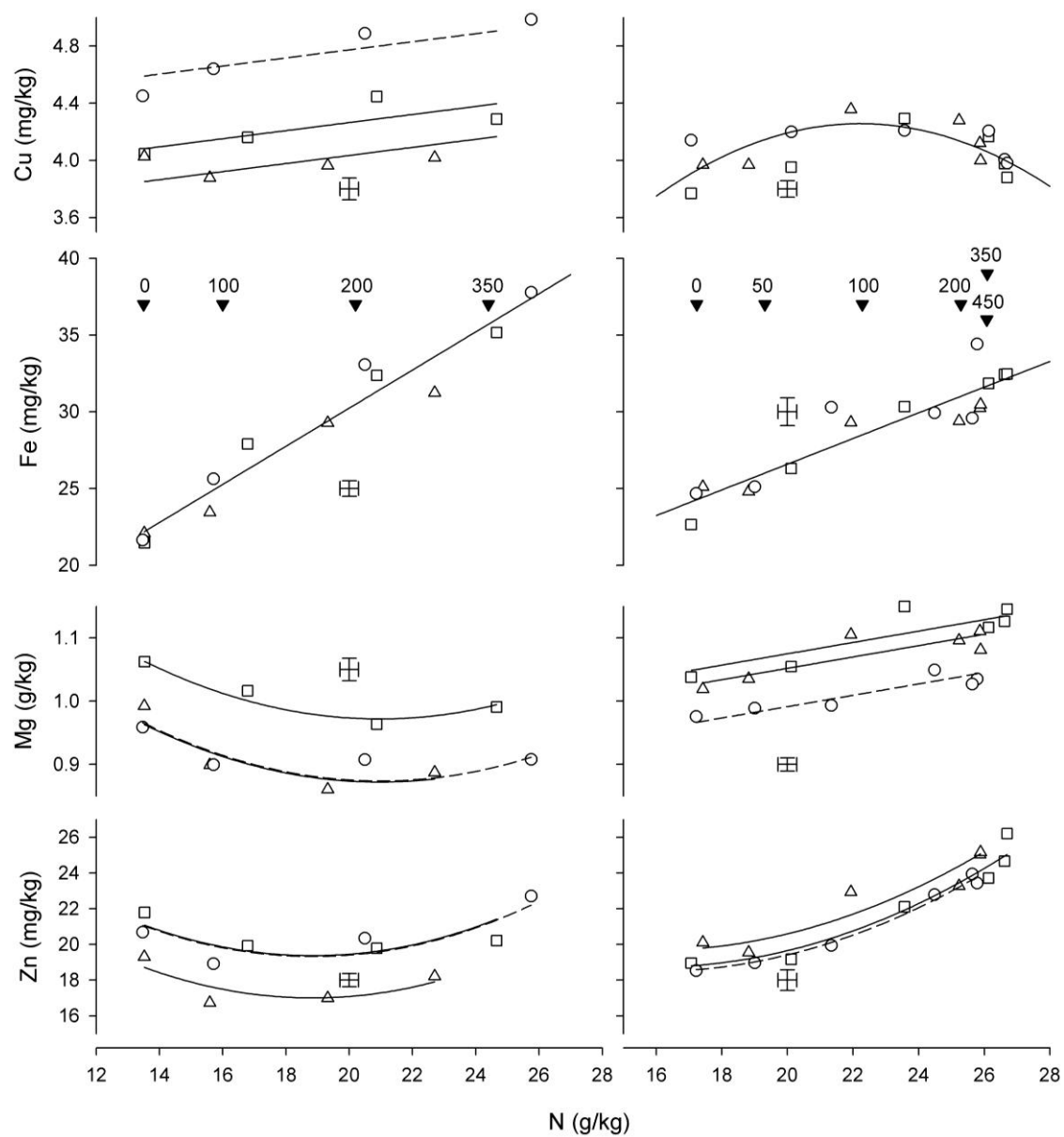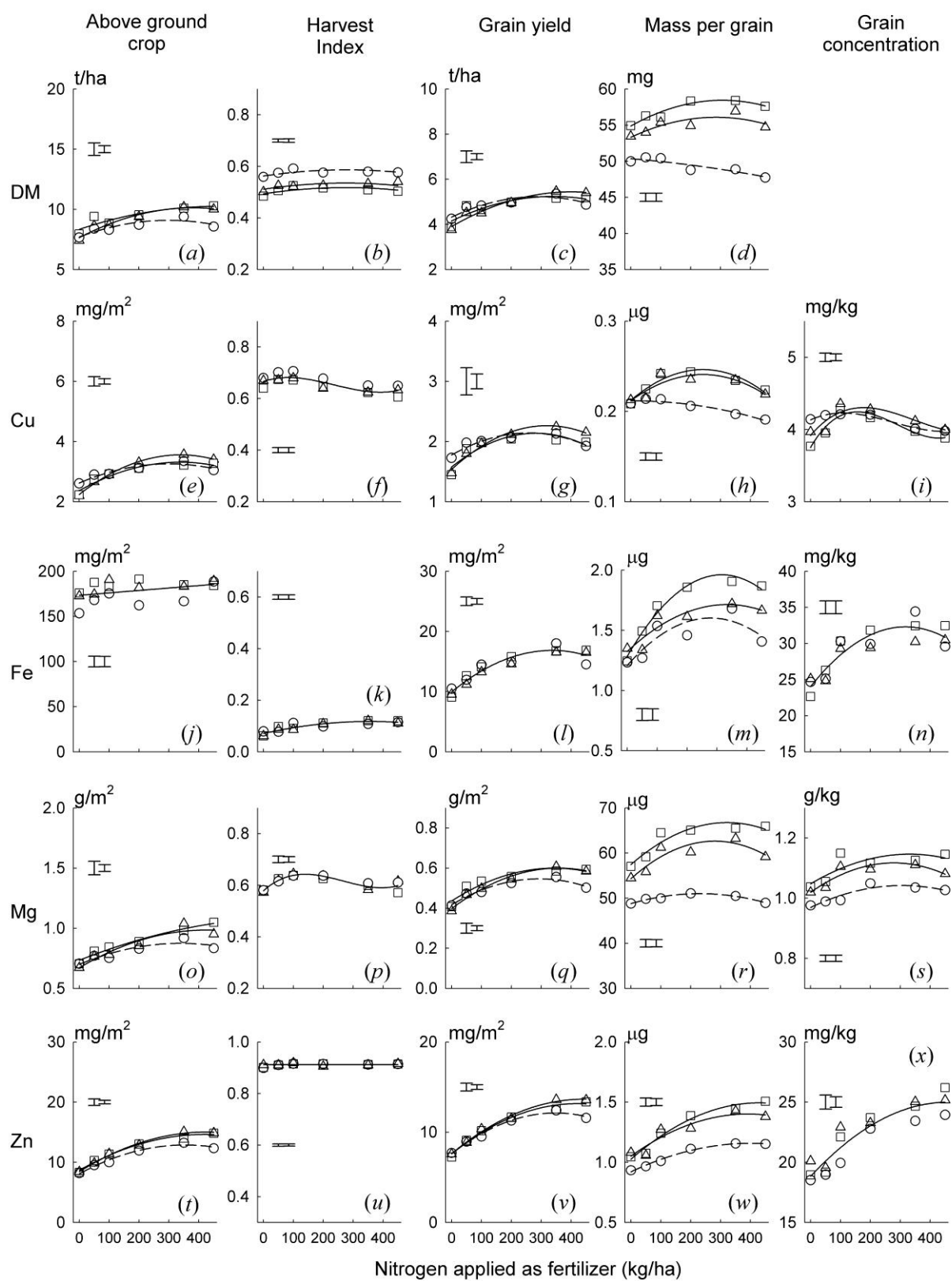
Above ground crop · Harvest Index · Grain yield · Mass per grain · Grain concentration

495

496   **Figure 1.**

497

Figure 2.

499

Above ground crop   Harvest Index   Grain yield   Mass per grain   Grain concentration

DM

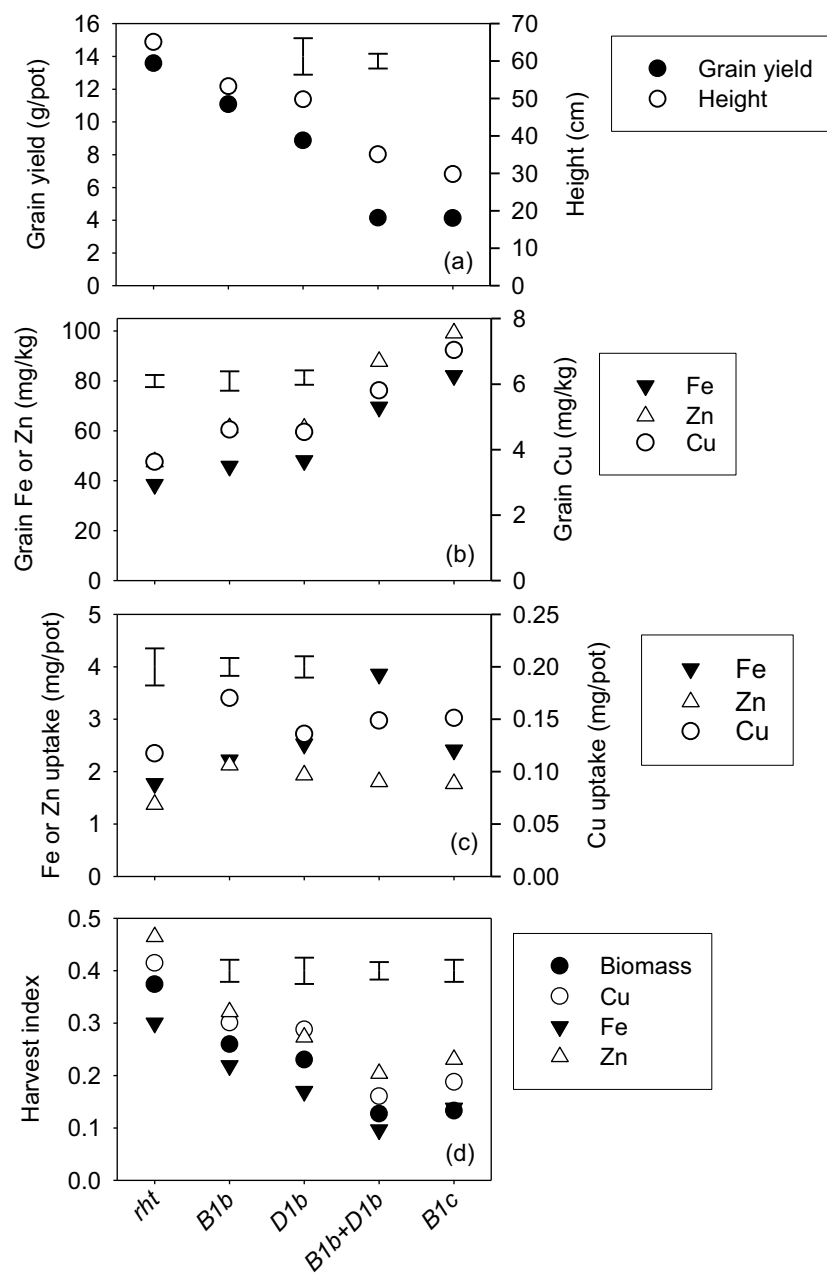Cu

Fe

Mg

Zn
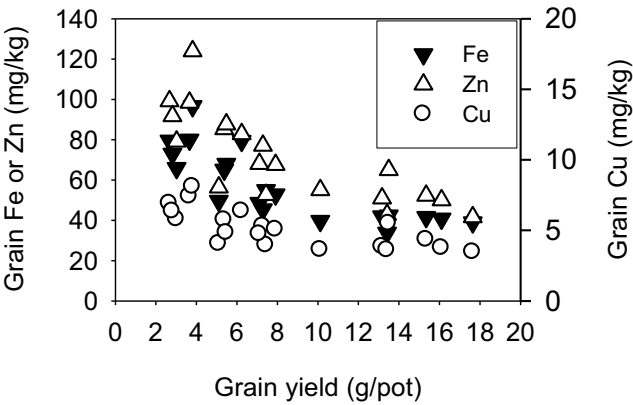
Nitrogen applied as fertilizer (kg/ha)

500

501   Figure 3.

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503   Figure 4.

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507   Figure 5.