

1 **Zinc fertilization increases productivity and grain nutritional quality of cowpea (*Vigna***
2 ***unguiculata* [L.] Walp.) under integrated soil fertility management**

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53 **ABSTRACT**

54 Cowpea (*Vigna unguiculata* [L.] Walp.) is an important but under-studied grain legume which
55 can potentially contribute to improved dietary zinc (Zn) intake in sub-Saharan Africa. In this
56 study, surveys were conducted on smallholder farms in Zimbabwe during 2014/15 to determine
57 the influence of diverse soil fertility management options on cowpea grain productivity and
58 nutrition quality. Guided by the surveys, field experiments were conducted to investigate the
59 influence of Zn fertilizer on the productivity and quality of cowpea under integrated soil fertility
60 management (ISFM). Experiments were conducted on two soil-types, namely, sandy (6% clay)
61 and red clay (57% clay) in 2014/15 and 2015/16 where cowpea was grown in rotation with staple
62 maize (*Zea mays* L.) and fertilized with combinations of Zn, nitrogen (N), phosphorus (P) and
63 two organic nutrient resources, cattle manure and woodland leaf litter. Cowpea grain yields on
64 surveyed farms ranged from 0.3 to 0.9 t ha⁻¹, with grain Zn concentration ranging from 23.9 to

65 30.1 mg kg⁻¹. The highest grain Zn concentration was on fields where organic nutrient resources
66 were applied in combination with mineral N and P fertilizers. Within the field experiments, mean
67 grain yields of cowpea increased by between 12 and 18% on both soil types when Zn fertilizers
68 were applied, from a baseline of 1.6 and 1.1 t ha⁻¹ on red clay and sandy soils, respectively. When
69 Zn fertilizers were co-applied with organic nutrient resources, grain Zn concentrations of cowpea
70 reached 42.1 mg kg⁻¹ (red clay) and 44.7 mg kg⁻¹ (sandy) against grain Zn concentrations of 35.9
71 mg kg⁻¹ and 31.1 mg kg⁻¹ measured in cowpea grown with no Zn fertilizer on red clay and sandy
72 soils, respectively. Agronomic biofortification of legumes is feasible and has the potential to
73 contribute significantly towards increasing dietary Zn intake by humans. A greater increase in
74 grain Zn on sandy than red clay soils under Zn fertilization illustrates the influence of soil type on
75 Zn uptake, which should be explored further in agronomic biofortification programs.

76

77 **Key words:** Agronomic biofortification; Dietary Zn supply; Grain legumes; Organic nutrient
78 resources; P-Zn interaction

79

80 1. INTRODUCTION

81 Zinc (Zn) is an essential micronutrient in both food crops and humans (FAO/IAEA/WHO, 2002).
82 Despite current increases in global food and energy supplies, Zn deficiency remains prevalent in
83 most developing countries (Cakmak et al., 2017) largely because the food systems in these
84 countries fail to supply adequate micronutrients (Gregory et al., 2017; Joy et al., 2014; Kumssa et
85 al., 2015; Manzeke et al., 2016). Symptoms of Zn deficiency in humans include impaired growth,
86 immuno-incompetence, pregnancy complications in child-bearing mothers, acute malnutrition
87 and otherwise curable diarrheal incidences in children under five years of age. These problems
88 continue to impose an economic burden in developing countries (FAO/WFP, 2002; Wessells and
89 Brown, 2012). Dietary Zn deficiency affects ~17% (1.1 billion people) of the global population
90 (de Valença et al., 2017; Kumssa et al., 2015; WHO, 2016). In sub-Saharan Africa (SSA) alone,
91 >25% of the population is at risk of inadequate dietary Zn intake compared with 9.6% in Central
92 and Eastern Europe (Wessells and Brown, 2012). The risk of Zn deficiency in Zimbabwe has
93 been estimated to be ~26%, based on food system supplies, but is likely to be greater among
94 some groups (Joy et al., 2015a; Kumssa et al., 2015).

95 Previous studies have shown that Zn-based fertilizers can improve dietary Zn supply in cereals
96 (Cakmak; 2008; Joy et al., 2015a; 2016; Wang et al, 2016; White and Broadley, 2009) by
97 increasing grain Zn concentration whilst simultaneously improving crop yields (Cakmak et al.,
98 2010; Welch and Graham, 2004; Zou et al., 2012). For example, Zn-based fertilizers have been
99 reported to increase productivity and nutritional composition of wheat (*Triticum aestivum* L.)
100 (Cakmak et al., 1999; Joy et al., 2016; Ram et al., 2016; Zou et al., 2012), maize (*Zea mays* L.)
101 (Harris et al., 2007; Manzeke et al., 2014; 2016) and rice (*Oryza sativa* L.) (Ram et al., 2016;

102 Shivay et al., 2015) grown on Zn-deficient soils. However, most studies on Zn fertilizer use have
103 largely focused on staple cereals with fewer such studies on grain legumes.

104 Grain legume crops support the livelihoods of poor households in SSA through contributing to
105 their dietary energy, protein and mineral intake (Messina, 1999; Mtambanengwe and Mapfumo,
106 2009; Rusinamhodzi et al., 2017). The average *per capita* consumption of grain legumes in
107 southern Africa is ~4.5 kg *capita*⁻¹ year⁻¹ (<http://www.fao.org/faostat/en/#data/FBS>). Grain
108 legumes have been reported to provide approximately 12% of dietary Zn supply (Joy et al.,
109 2014), although there is considerable variation between countries. In Zimbabwe, of the 10 mg Zn
110 *capita*⁻¹ day⁻¹ supplied by major foods, grain legumes provide only 10% (1.0 mg Zn *capita*⁻¹ day⁻¹
111 ¹) compared to a supply of up to 8.7 mg Zn *capita*⁻¹ day⁻¹ in West Africa (Joy et al., 2014). An
112 example of an important drought tolerant grain legume under smallholder cropping in SSA is
113 cowpea (*Vigna unguiculata* [L.] Walp). Despite its exceptional biological nitrogen fixing (BNF)
114 potential on nutrient-depleted soils and a relatively high protein content of up to 25% (IITA,
115 2015; Rusinamhodzi et al., 2006), the productivity of cowpea has increasingly declined in part,
116 due to lack of nitrogen (N) and phosphorus (P) fertilization (Giller, 2001; Kanonge et al., 2015;
117 Zingore et al., 2008).

118 Research on Zn fertilizer use in grain legumes has mostly been done under greenhouse conditions
119 (Brennan et al., 2001; Poblaciones and Rengel, 2016; Valenciano et al., 2010), with limited
120 studies at field and farm levels (e.g. Johnson et al., 2005; Khan et al., 2000). To date we are not
121 aware of studies exploring the optimal use of Zn fertilizers in the context of the integrated soil
122 fertility management (ISFM) approaches, which encompass organic nutrient resource use and
123 appropriate rotations in grain legume production, yet this is how farmers are encouraged to grow
124 crops on nutrient-depleted sandy soils of southern Africa (Giller, 2001; Kanonge et al., 2015;

125 Mapfumo et al., 2001; Mpeperekwi et al., 2000; Mtambanengwe and Mapfumo, 2009). The
126 legume-cereal rotations help build soil fertility, diversify household diets and break crop pests
127 and disease cycles.

128 Application of N fertilizers promotes uptake and translocation of Zn and other micronutrients
129 (Aciksoz et al., 2011) in wheat (Kutman et al., 2010; 2011) and rice (Jaksomsak et al., 2017),
130 whereas P fertilizer application decreases Zn uptake in dwarf bean (*Phaseolus vulgaris* L., cv.
131 Borlotto nano) due to a dilution effect (Alloway, 2008; Gianquinto et al., 2000; Prasad et al.,
132 2016; Zhu et al., 2001). However, N x Zn, and P x Zn interaction effects on nutrition of field-
133 grown grain legumes have not been reported previously. The objectives of this study were: i) to
134 determine grain yield and grain Zn nutritional quality of cowpea grown on smallholder farms
135 under diverse soil fertility management options used by farmers; ii) to determine the productivity
136 and grain quality of cowpea fertilized with combinations of Zn-, N- and P-based fertilizers and
137 locally available organic nutrient resources grown under a cowpea-maize rotational sequence; iii)
138 to evaluate the potential contribution of Zn-fertilized cowpea towards dietary Zn supplies for
139 households reliant on legume-cereal rotational systems.

140 **2. MATERIALS AND METHODS**

141 The study was conducted in Hwedza District (18° 41' S, 31° 42' E) in Eastern Zimbabwe. It
142 comprised a survey of 60 farmers in 2014/15, and field experiments at two sites in 2014/15 and
143 2015/16 cropping seasons. The study builds on the Soil Fertility Consortium for Southern Africa
144 (SOFECSA)'s work on legume production in smallholder farming communities under diverse
145 ISFM techniques that included systematic legume-cereal rotations, crop diversification and
146 combined use of mineral and organic nutrient resources. SOFECSA had been working with
147 smallholder farmers in Hwedza since 2005. Hwedza encompasses three of Zimbabwe's agro-

148 ecological region/natural regions (NR) IIb to IV, receiving 450-800 mm year⁻¹ between
149 November and March. Soils in this community are broadly classified as Lixisols
150 (FAO/ISRI/ISSS, 2006). Maize is the dominant crop under a mixed crop-livestock farming
151 system (Mtambanengwe and Mapfumo, 2009). Legumes such as groundnut (*Arachis hypogea*
152 L.), cowpea and common bean (*Phaseolus vulgaris* L.) are typically grown on smaller patches of
153 land compared with the staple maize (Rusinamhodzi et al., 2006), often with minimal or zero
154 fertilization (Kanonge et al., 2015) resulting in inefficient legume-cereal rotational systems.
155 Cattle are the dominant livestock mainly kept for manure and draught power provision. In the
156 absence of cattle manure, farmers often collect woodland leaf litter from the tropical savanna
157 woodlands for soil fertility management. Rainfall in Hwedza is often uneven (Rurinda et al.,
158 2013), for example, the district received >800 mm annum⁻¹ in the 2014/15 cropping season, with
159 314 mm obtained within the month of December 2014 alone (Figure 1).

160 **2.1 Survey**

161 A survey was conducted in Dendenyore (agro-ecological zone IIb) and Ushe (agro-ecological
162 zone III-IV) Wards in Hwedza to determine the range of soil fertility management options
163 employed under cowpea production and to quantify grain yields and Zn nutritional composition.
164 The survey targeted households working with SOFECSA on cowpea production, and other grain
165 legumes, under its ISFM initiatives. Farmers (n=60) were selected randomly from a total of 150
166 farmers under the SOFECSA cowpea production initiative with the help of local Agricultural
167 Extension Workers (AEWs). Under the SOFECSA program, one main variety of cowpea, CBC2,
168 which is a high-yielding, semi-bushy, short season (60-90 days to maturity) cultivar, has been
169 promoted to eliminate genotypic variation. The farmers planted and managed the cowpea using
170 agronomic recommendations appropriate within their agro-ecological zones (AGRITEX, 1985),

171 with technical support from AEWs and SOFECSA researchers. Appropriate agronomic
172 recommendations included plant spacing of 0.45 m x 0.075 m and application of agro-chemicals
173 to control aphid manifestation during the hot and dry periods of the cropping season. Research
174 approval for this study was obtained from the Department of Agricultural Technical and
175 Extension Services (AGRITEX) of The Government of Zimbabwe's Ministry of Agriculture,
176 Mechanization and Irrigation Development.

177 ***2.1.1 Determination of farmer soil fertility management options and cowpea grain yields***

178 The amount of mineral fertilizer and organic nutrient resources used by farmers on cowpea were
179 quantified by direct measurements in farmers' fields. This was supported by data collected
180 through a pre-tested questionnaire by interviewing the host farmers. In some cases the amounts
181 were given in local units and then converted to kg ha⁻¹. For example, a standard bucket and
182 scotch cart of cattle manure or woodland leaf litter measured ~20 and 350 kg, respectively.
183 Cowpea grain yield was quantified at physiological maturity from three replicate plots within a
184 field, with each plot measuring 9 m². The cowpea fields measured between 0.05-0.4 ha.
185 Harvested cowpea pods were air-dried, shelled and grain yield determined at 9.5% moisture
186 content. A subsample of ~100 g was ground through a 0.5 mm sieve in a stainless steel Thomas-
187 Wiley Model 4 Laboratory mill (Thomas Scientific, Swedesboro, USA) for elemental analysis of
188 Zn.

189 ***2.1.2 Selection of fields for experimentation***

190 Soil samples were collected from each cowpea field and analyzed for Zn to guide selection of
191 field experimental sites. A composite soil sample was collected from 10 random points in each
192 field at a depth of 0-20 cm using a Dutch auger. Soil samples were air-dried, sieved through a 2

193 mm stainless steel sieve and ground to $<40\ \mu\text{m}$ in an agate Retsch PM400 Planetary Ball Mill
194 (Haan, Germany). The samples (0.25 g) were digested as described in Joy et al. (2015b) for a
195 broad suite of trace and major elements including total P and Zn in a mixed acid solution (HF 2.5
196 mL:HNO₃ 2 mL:HClO₄ 1 mL:H₂O₂ 2.5 mL). Subsequent total elemental analyses of the acid
197 digests was carried out by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS; Agilent
198 7500cx, Santa Clara, USA) in collision cell gas mode (He gas) as described in Hamilton et al.
199 (2015) and Joy et al. (2015b).

200 A portion of the sieved ($\text{Ø}<2\ \text{mm}$), un-milled soil samples were analyzed for soil texture, pH,
201 available P, total N and exchangeable bases (calcium-Ca²⁺, magnesium-Mg²⁺ and potassium-K⁺)
202 using standard protocols as described by Anderson and Ingram (1993). Extractable Zn was
203 determined using the ethylene diamine tetra-acetic acid (EDTA) method (Norvell, 1989). The
204 concentration of Zn²⁺ was determined by atomic absorption spectroscopy (AAS) using a Varian
205 SpectrAA 50 spectrophotometer (Varian Pvt Ltd, Mulgrave, Australia). Soil organic matter
206 content was determined by loss-on-ignition (LOI) at 450°C, in an Elite Thermal muffle furnace
207 (Model BCRF 12/13-2416, Market Harborough, UK), for 1 g of ($\text{Ø}<40\ \mu\text{m}$) soil (Joy et al.
208 2015b). Certified Reference Materials (CRMs) used for quality assurance were BGS 102
209 (Ironstone soil, British Geological Survey-NERC, Nottingham, UK) and NIST 2711 (Montana
210 soil, US Geological Survey-National Institute of Standards and Technology, Virginia, USA).
211 Measurements for total P and Zn of soil CRMs by ICP-MS provided performance characteristics
212 of $101 \pm 5\%$ and $96 \pm 7.9\%$, for P and Zn, respectively ($n = 12$) for BGS 102 and $101 \pm 6.7\%$ and 93
213 $\pm 4.9\%$ for P and Zn, respectively ($n = 6$) for NIST 2711. The majority of the fields ($>70\%$) had a
214 EDTA extractable soil Zn status of below $1.5\ \text{mg kg}^{-1}$, indicating that the soil was low/deficient
215 in Zn (Dobermann and Fairhurst, 2000; Zare et. al., 2009).

216 Based on the results of the preliminary soil analyses, two experimental field sites of contrasting
217 soil physical (texture) and chemical properties were selected in Dendenyore Ward: a sandy soil
218 (18°41'45.72" S; 31°41'28.49" E) and red clay soil (18°42'24.58" S; 31°41'54.30" E). The sites had
219 a low (sandy soil, 0.98 mg kg⁻¹) to adequate (red clay soil, 1.70 mg kg⁻¹) (Table 1) plant available
220 soil Zn status and represented different categories of soil type where cowpea is usually grown on
221 smallholder farms in Zimbabwe, with the sandy soils representing a greater proportion of the
222 surveyed fields. The underlying rationale was that soil texture could potentially influence
223 fertilizer uptake. Both field sites were under an unfertilized cowpea crop during the preceding
224 cropping seasons. It is a common practice by smallholder farmers in Zimbabwe, and elsewhere in
225 southern Africa, to grow grain legumes without any fertilizer input (Kanonge et al., 2015; Snapp
226 et al., 2002).

227 **2.2 Field experiments**

228 ***2.2.1 Determination of experimental treatments***

229 Experimental treatments to examine the value of Zn fertilization on cowpea productivity and
230 grain Zn were designed to augment existing farmer practices (Table 2). Guided by earlier
231 SOFECSA research (Kanonge et al., 2015; Mtambanengwe and Mapfumo, 2009;
232 Mtambanengwe et al., 2015) and the range of ISFM practices from the surveyed farms, a
233 cowpea-maize rotational sequence comprising 10 treatments was tested. An incomplete factorial
234 treatment design was used with four cowpea treatments and six maize treatments in the 1st season
235 which were rotated in the 2nd cropping season (Table 2). Each treatment was replicated three
236 times, and plot sizes measured 4.5 m x 5 m in gross area.

237 The treatments fell into two broad categories: 1. Mineral fertilizers only and, 2. Combinations of
238 organic and mineral fertilizers. These are given in Table 2. To represent an appropriate ISFM
239 technique, treatments simulated a cowpea and maize rotational system. Treatments under maize
240 during the 1st season were grown to cowpea in the 2nd year. Maize treatments were informed by
241 earlier work on influence of farmer management and organic nutrients on grain Zn nutrition
242 under smallholder maize cropping (Manzeke et al., 2012; 2014).

243 To ensure Zn was the only nutrient limiting growth, the mineral fertilizer category had a positive
244 control treatment without Zn, which supplied N and P at 90 kg N ha⁻¹ + 26 kg P ha⁻¹ to maize,
245 and 30 kg N ha⁻¹ + 26 kg P ha⁻¹ to cowpea. Despite >50% of the surveyed farmers not using
246 fertilizers on cowpea, the majority of soils on smallholder farms in Zimbabwe are inherently N
247 and P deficient (Grant, 1981), which limits crop productivity. To eliminate N and P deficiencies
248 under both the maize and cowpea, we applied recommended N and P in the control treatments
249 over the two cropping seasons. Starter N is required to “kick-start” legume productivity under
250 such poor soils (Kanonge et al., 2015) and it is known to improve micronutrients accumulation in
251 grains (Gregorio et al., 2000; Kutman et al., 2011). Phosphorus is not only important for
252 enhancing biological nitrogen fixation (BNF) under nutrient-depleted soils (Giller, 2001), but
253 also for increasing yields of grain legumes (Mapfumo et al., 2001; Zingore et al., 2008). The
254 cowpea crop received a third of the N fertilizer in both seasons because we assumed it derives its
255 N from BNF as well as benefit from residual soil N from season 1. However, we maintained the
256 levels of P fertilization in both cowpea and maize across the two cropping seasons.

257 Guided by earlier SOFECSA work (Kanonge et al., 2015; Manzeke et al., 2014), maize received
258 10 t organic material ha⁻¹ in the 1st year while cowpea received 5 t ha⁻¹. Of the commonly
259 available organic nutrients on-farm (i.e. compost, woodland leaf litter and cattle manure), we

260 only tested cattle manure on cowpea in the 1st season as it is mostly used by farmers (also see
261 Kanonge et al., 2015), but on maize we had treatments with woodland leaf litter and cattle
262 manure because they are the dominant organic nutrients used in maize production (Manzeke et
263 al., 2012). Despite use of sole organic nutrients in cowpea production by some of the surveyed
264 farmers, we deliberately did not include this option because of low P levels in most of the organic
265 nutrient resources, especially cattle manure (Murwira et al., 1995). The low mineral N (16 kg ha⁻¹
266 ¹) and P (14 kg ha⁻¹) treatment, co-applied with locally available cattle manure, was included to
267 cater for farmers who often fail to supply optimal mineral fertilizer to their legume crops. Zinc
268 and organic nutrient resources were only applied in the 1st year of cropping because their residual
269 fertility benefits last up to three cropping seasons (Cakmak, 2008; Mtambanengwe and
270 Mapfumo, 2005).

271

272 ***2.2.2 Establishment and management of the experiment***

273 Land was prepared by conventional ploughing, using an animal-drawn mould-board plough, to a
274 fine tilth before application of fertilizers and planting. Compound D (7N:14 P₂O₅:7K₂O),
275 elemental Zn (applied as ZnSO₄·7H₂O with 22% Zn) and organic nutrient resources were
276 broadcast at planting and then incorporated into the soil by hand hoe. The cattle manure
277 contained 24% organic C, 0.9% N and 29.6 mg Zn kg⁻¹ dry weight. Woodland leaf litter had a
278 relatively higher organic C, N and Zn concentrations of 37%, 1.2% and 79.8 mg kg⁻¹,
279 respectively. Thus, application of 10 t ha⁻¹ dry weight of the two organic nutrient resources
280 supplied approximately 296 g Zn ha⁻¹ (cattle manure) and 798 g Zn ha⁻¹ (woodland leaf litter).
281 The total amount of Zn added from organic nutrient resources and mineral Zn fertilizer over the
282 two year cropping period is shown in Table 2. Mineral N and P were supplied to the cowpea crop

283 solely as a basal fertilizer (Compound D) except when applied in combination with organic
284 nutrient resources (see Table 2). For the maize crop, planted at a population density of ~37,000
285 plants ha⁻¹, ammonium nitrate (AN; 34.5%N) was applied as top dressing in three splits of 30%,
286 40% and 30% at 2 weeks after emergence (WAE), 6 WAE and at silking, respectively. Cowpea
287 (CBC2) was planted at a spacing of 0.45 m x 0.075 m in triplicate plots measuring 22.5 m² to
288 achieve a population of ~296,000 plants ha⁻¹. Weeding was done manually using hand-hoes at 3
289 and 6 weeks after crop emergence (WAE), resulting in effective control of weeds throughout the
290 growing season. Rogor (Dimethoate 50 EC, Agricura, Harare, Zimbabwe) was used to control
291 aphids in cowpea at a rate of 300 mL ha⁻¹.

292 ***2.2.3 Plant shoot biomass and grain yield quantification***

293 Above ground cowpea shoot biomass was quantified at flowering during both cropping seasons
294 using 0.25 m² quadrats, from three random sampling points per plot on the sandy soil site. No
295 biomass was collected from the red clay experimental site during the 1st cropping season due to
296 poor germination. The biomass yield was determined on a dry matter basis after oven-drying at
297 60°C to constant weight. Cowpea and maize grain yields were quantified at physiological
298 maturity from a net plot measuring 10.8 m², at a moisture content of 9.5 and 12.5%, respectively.
299 Dried grain samples were ground in a Thomas-Wiley Model 4 Laboratory mill (Thomas
300 Scientific, Swedesboro, USA) to pass through a 0.5 mm sieve. All crop (maize/cowpea) residues
301 were left on the field surfaces, and consumed by livestock during the dry season.

302 .

303 **2.3 Elemental analysis of grain samples from farmers' and experimental fields**

304 The finely ground cowpea grain samples from 1st season experimental plots and selected sample
305 duplications from the farmers' fields were ashed, digested with aqua regia (1 HNO₃: 3HCl)
306 solution and analyzed for total Zn and P using an AAS. Plant Certified Reference Materials
307 (CRMs) used were NIST 1573a (Tomato leaf; National Institute of Standards and Technology,
308 Virginia, USA) and NIST 1567b (Wheat flour; National Institute of Standards and Technology).
309 Colorimetric measurements for P on VIS spectrophotometer and Zn by AAS of plant CRMs
310 provided performance characteristics of 96.7 ±1.9% and 99.6 ±3.1%, for P and Zn, respectively
311 for NIST 1573a and 95.2 ±2.3% and 94.7 ±2.6% for P and Zn, respectively for NIST 1567b.

312 Grain samples from the 2nd cropping season, CRMs (NIST 1570a, spinach leaves and NIST
313 1573a tomato leaves; National Institute of Standards and Technology) and blanks were analysed
314 for multi-elements including total Zn and P using a mixed acid (HNO₃ 10mL:H₂O₂ 1mL) solution
315 in a closed vessel microwave heating system (MARS Xpress, CEM Corporation, Matthews,
316 United States) as described by Joy et al. (2015b). Each analysis of 20 samples included two
317 reagent blank samples, random sample duplications and CRMs for quality control. Subsequent
318 total elemental analysis was carried out by ICP-MS. Performance characteristics for NIST 1570a
319 of 108 ±8.0% and 91.9 ±7.4% for P and Zn, respectively, and 106 ±9.0% and 90.4 ±5.1% for P
320 and Zn, respectively, for NIST 1573a were obtained. To validate elemental Zn and P analysis
321 results obtained using AAS, selected cowpea grain samples from 1st season experimental plots
322 and farmers' fields were re-analyzed using the ICP-MS, and the results were comparable.

323 Nutrient uptake (g ha⁻¹) was quantified on a dry weight basis as the product of nutrient
324 concentration in the grain (mg kg⁻¹) and grain yield (t ha⁻¹).

325 To estimate Zn bioavailability in humans, the PA to Zn molar ratio was estimated using a 65%
326 grain P conversion ratio (O'Dell et al., 1972; Wu et al., 2009). The subsequent estimated PA:Zn

327 molar ratio was calculated by dividing PA by grain Zn concentration. Zinc absorption is often
328 inhibited by high phytate in grains, a major storage of P which is not digested by monogastric
329 animals including humans (Azeke et al., 2011; Lönnerdal, 2000). A PA:Zn molar ratio >15-20 is
330 considered to hinder efficient absorption of Zn in the digestive tract (Gibson, 2007; Morris and
331 Ellis, 1989).

332 **2.4 Survey and experimental data analyses**

333 Data from the survey and field experiments were tested for normality before being subjected to
334 analysis of variance (ANOVA) using GENSTAT 18th Edition (VSN Scientific, Hemel
335 Hempstead, UK). The Fisher's least significant difference (LSD) test was used to compare
336 cowpea biomass, grain yield, grain nutritional value (Zn, P, estimated PA:Zn) and Zn uptake
337 treatment means at probability $P < 0.05$. To assess the added crop yield benefits from Zn
338 fertilization, percentage differences (positive or negative gain) in yield were calculated using
339 previous cowpea yield data on similar soils with no addition of Zn fertilizers. A daily cowpea
340 consumption of $100 \text{ g person}^{-1} \text{ day}^{-1}$ (Pereira et al., 2014; Petry et al., 2015) and a recommended
341 adult daily Zn intake of $14 \text{ mg person}^{-1} \text{ day}^{-1}$ which assumes a typical low Zn bioavailability diet
342 (WHO/FAO, 2004), were used to calculate and benchmark the potential dietary contribution of
343 each fertilization option to Zn nutrition.

344 **3. RESULTS**

345 **3.1 Farmer soil fertility management options and their influence on cowpea productivity** 346 **and grain Zn**

347 The crop survey revealed that more than half of the farmers did not apply any form of mineral
348 fertilizers or organic nutrients to their cowpea crop (Table 3). One third of the farmers applied

349 basal mineral N and P fertilizer at planting, with fertilizer rates ranging from 3.5-30 kg N ha⁻¹
350 (mean = 14) and 0.3-26 kg elemental P ha⁻¹ (mean = 8) (Table 3). The fertilizer
351 amounts/quantities applied to cowpea varied by farmer resource endowment. Only 11% of the
352 farmers applied organic nutrient resources in the form of cattle manure, woodland leaf litter or
353 composts, either alone (3%) or in combination with mineral fertilizers (8%). These differences in
354 fertilizer and ISFM strategies by smallholder farmers resulted in differences in grain yield
355 (P<0.05) and grain Zn concentration and uptake (P<0.01) (Table 4). The largest mean cowpea
356 grain yield of 895 kg ha⁻¹ (range = 400-1000 kg ha⁻¹) was obtained when mineral fertilizers and
357 organic nutrients were used in combination. Yields were less when organic nutrient resources
358 (mean=683 kg ha⁻¹) or mineral NPK treatments (mean=566 kg ha⁻¹) were used alone (Table 4).
359 Unfertilized crops gave mean grain yields of less than 300 kg ha⁻¹ (range = 40-600 kg ha⁻¹).
360 The highest cowpea grain Zn concentration of 30.1 mg kg⁻¹ was observed when organic nutrients
361 were used in combination with mineral fertilizer, and this corresponded to a grain Zn uptake of
362 26.9 g ha⁻¹ (Table 4). When organic nutrients were used alone, grain Zn concentration was 27.7
363 mg kg⁻¹ and grain Zn uptake was 18.9 g Zn ha⁻¹. The mineral fertilized and the unfertilized crops
364 had the lowest grain Zn concentrations of 24.4 and 23.9 mg kg⁻¹, respectively, and Zn uptakes of
365 13.8 and 6.8 g Zn ha⁻¹, respectively.

366

367 **3.2 Contribution of Zn and ISFM to cowpea grain yields**

368 Yields obtained from the field experiments were consistently higher than those under smallholder
369 cropping in the survey (<0.6 t ha⁻¹; Table 4). During the 1st season, cowpea grain yields averaged
370 1.5 t ha⁻¹ (range=1.1-1.8 t ha⁻¹) and 1.2 t ha⁻¹ (range=0.8-2.0 t ha⁻¹) on the red clay (Figure 2a)

371 and sandy soil (Figure 2b), respectively. Zinc fertilizer application did not significantly influence
372 grain yields on the red clay soil ($P>0.05$; Figure 2a). However, on the sandy soil, application of
373 Zn significantly ($P<0.01$) increased grain yields by $\sim 0.2 \text{ t ha}^{-1}$ (18%) (Figure 2b). The
374 combination of organic cattle manure, Zn and high rates of mineral N and P increased cowpea
375 grain yields by 38% on the red clay soil and more than doubled to 2 t ha^{-1} on the sandy soil. On
376 the red clay soil, co-application of cattle manure, Zn and a low rate of mineral N and P resulted in
377 cowpea grain yields of 1.7 t ha^{-1} (Figure 2a). Despite a lack of significant differences in cowpea
378 grain yields on the red clay soil, these results were, however, greater than yields of treatments
379 receiving mineral N, P and Zn without cattle manure, which yielded 1.3 t ha^{-1} (Figure 2a).

380 On the sandy soil, such increases in grain yields with cattle manure use were not evident when Zn
381 was co-applied with lower rates of mineral N (16 kg ha^{-1}) and P (14 kg ha^{-1}) (Figure 2b). For
382 example, with Zn fertilizer application, cowpea grain yields of 1.0 t ha^{-1} were measured under
383 the lower rate of mineral + cattle manure treatment, and these were comparable to yields of 0.9 t
384 ha^{-1} when Zn was co-applied with highest rates of mineral N and P alone (Figure 2b).

385 In the 2nd year of cropping, when cowpea followed maize, grain yields ranged between $1.6\text{-}1.9 \text{ t}$
386 ha^{-1} and $1.1\text{-}1.4 \text{ t ha}^{-1}$ on the red clay (Figure 2c) and sandy soils (Figure 2d), respectively.

387 Treatments receiving mineral N and P alone without Zn consistently gave the lowest yields of 1.6
388 and 1.1 t ha^{-1} on red clay and sandy soils, respectively. There was no effect of Zn application on
389 grain yields on the clay soil ($P>0.05$; Figure 2c). On the sandy soil, application of Zn
390 significantly ($P<0.001$) increased grain yields by 18% compared to plots receiving sole mineral N
391 and P (Figure 2d). On the same site, application of Zn increased grain yields by 16% in plots
392 receiving both mineral and organic (woodland leaf litter) inputs compared to plots receiving
393 mineral and organic woodland leaf litter without Zn (Figure 2d). When Zn was applied to mineral

394 + organic treatments, the woodland leaf litter treatment significantly out-performed the cattle
395 manure treatment by 0.1 t on the sandy soil. However, comparable yields were attained between
396 the two organic nutrients when Zn was applied in combination with mineral N and P on the red
397 clay soil. Despite higher average yields on red clay soil than on sandy soil, rotation effects and
398 residual fertility benefits of Zn and organic nutrients on grain yield were more apparent on the
399 sandy soil (Figure 2d) which consistently gave significantly different cowpea grain yields among
400 treatments than on the red clay soil (Figure 2c). On both soil types, there was a tendency of
401 increased cowpea grain yields in the 2nd year of cropping under the sole mineral treatments with
402 or without Zn compared to yields attained under the same treatments during the 1st year of
403 experimentation (Figure 2). On the sandy soil, apparently lower cowpea grain yields averaging
404 1.3 t ha⁻¹ were observed in treatments with combined applications of 10 t ha⁻¹ organic nutrient
405 resources and mineral fertilizer (Figure 2d) compared with average yields of 1.5 t ha⁻¹ in the 5 t
406 ha⁻¹ cattle manure treatments (Figure 2b) obtained during the 1st season.

407

408 **3.3 Effect of Zn on cowpea establishment and shoot biomass yield**

409 On the sandy soil, fertilization of cowpea with Zn increased shoot biomass productivity by 6% in
410 the 1st season (Figure 3a) and by between 20% and 35% relative to the non-Zn control which
411 yielded 1.9 t ha⁻¹ in the 2nd season (Figure 3b). Further significant increases in shoot biomass
412 were observed when Zn was applied in combination with organic and mineral fertilizers ($P < 0.05$;
413 Figure 3b). For example, during the second year of cropping, cowpea biomass yields on the
414 sandy soil reached 2.7 t ha⁻¹ when Zn was applied with woodland leaf litter, compared to 2.4 t ha⁻¹
415 when Zn fertilizers were applied to the solely mineral N + P treatments. In the same year, no

416 significant differences ($P>0.05$) in cowpea biomass yields were attained on the red clay soil
417 (Figure 3c).

418 **3.4 Influence of Zn fertilization, organic nutrient resource use and mineral N and P on** 419 **cowpea grain nutritional quality**

420 *3.4.1 Effect of fertilization on cowpea grain Zn and uptake*

421 Grain Zn concentrations were generally greater in crops grown on the red clay soil than on sandy
422 soil. Grain Zn concentration ranged from 29.2-40.2 mg kg⁻¹ on the red clay soil and 18.5-30.2 mg
423 kg⁻¹ on the sandy soil during the 1st season (Table 5). Greater grain Zn concentrations, of between
424 35.9-42.1 mg kg⁻¹ and 31.1-44.7 mg kg⁻¹, were observed on the red clay and sandy soils,
425 respectively, during the 2nd season (Table 6). Grain Zn uptake ranged from 30.9-70.3 g ha⁻¹ and
426 14.8-53.1 g ha⁻¹ on the red clay and sandy soils, respectively, during the 1st season (Table 5).
427 Higher Zn uptake was observed during the 2nd season, ranging from 57.4-78.2 g ha⁻¹ and 34.2-
428 64.7 g ha⁻¹ on the red clay and sandy soils, respectively. On the sandy soil, application of Zn
429 significantly increased grain Zn concentration ($P<0.01$) and uptake ($P<0.05$). However, there
430 were no significant effects ($P>0.05$) of Zn application on grain Zn concentration and uptake on
431 the clay soil.

432 During the 1st season, control plots receiving solely mineral N and P had grain Zn concentrations
433 of 29.2 and 18.5 mg kg⁻¹ on the red clay and sandy soils, respectively (Table 5). Grain Zn
434 concentration was proportionally more responsive to Zn fertilizers and organic matter on sandy
435 soils than on red clay soil. When Zn was applied to the solely mineral N and P treatment, grain
436 Zn concentration did not increase significantly on red clay soil, but increased to 24.8 mg kg⁻¹ on
437 sandy soil. The greatest grain Zn concentrations were observed (40.2 mg kg⁻¹ on red clay soil;

438 30.2 mg kg⁻¹ on sandy soil) when cattle manure and Zn fertilizers were combined with lower
439 mineral N (16 kg ha⁻¹) and P (14 kg ha⁻¹) rates. At higher N (30 kg ha⁻¹) and P (26 kg ha⁻¹) rates
440 combined with cattle manure and Zn applications, grain Zn was 37.4 mg kg⁻¹ and 26.2 mg kg⁻¹ on
441 the red clay and sandy soils, respectively.

442 During the 2nd season, the highest cowpea grain Zn concentrations of up to 42.1 mg kg⁻¹ on clay
443 and 44.7 mg kg⁻¹ on sandy soil were measured under the treatment that combined woodland leaf
444 litter with mineral N, P and Zn (Table 6). However, there were no significant treatment
445 differences (P>0.05) in grain Zn concentration on the red clay soil, with significant treatment
446 differences only apparent on the sandy soil (P<0.01). On both soils, the greatest grain Zn
447 concentrations were observed when residual woodland leaf litter and Zn fertilizer were co-
448 applied with 30 kg N ha⁻¹ and 26 kg P ha⁻¹, translating to 7% and 39% higher grain Zn compared
449 to the non-Zn woodland leaf litter treatment on the red clay and sandy soils, respectively. The
450 woodland leaf litter with Zn treatments resulted in 2% and 16% more grain Zn concentration than
451 the cattle manure + Zn treatment on the red clay and sandy soils, respectively (Table 6). Up to
452 16% and 40% more grain Zn concentration was measured when mineral N and P was applied on
453 treatments with residual Zn fertility on the red clay and sandy soils, respectively compared to
454 plots receiving sole mineral N and P. All sole mineral N and P without Zn treatments consistently
455 had the lowest grain Zn concentrations of 35.9 and 31.1 mg kg⁻¹ on the red clay and sandy soils,
456 respectively.

457 ***3.4.2 Effect of fertilization on grain P and the phytic acid:Zn molar ratio in cowpea***

458 There were no apparent differences in cowpea grain P concentration between the red clay and
459 sandy soils. During the 1st season, grain P concentration ranged from 3.0-3.8 g kg⁻¹ (mean 3.3)
460 and 1.9-3.2 g kg⁻¹ (mean = 2.7) on red clay and sandy soils, respectively, (Table 5). During the

461 2nd season, grain P ranged from 2.6-3.1 (mean = 2.8) and 3.0-3.3 (mean = 3.2) on clay and sandy
462 soils, respectively (Table 6). These high grain P concentrations are likely to translate to high
463 PA:Zn molar ratios. During the 1st season, estimated PA:Zn ranged from 50.1-84.6 (mean=63.1)
464 on the red clay soil and 47.1-112.4 (mean=72.7) on the sandy soil. During the 2nd season, lower
465 mean PA:Zn ratios of 46.2 and 57.7 were observed on the red clay and sandy soils, respectively
466 (Table 6).

467 The estimated PA:Zn molar ratios are smaller in grains with greater Zn concentrations, for
468 example, those grown on clay soils and those fertilized with Zn and organic matter (Table 6). The
469 solely mineral N and P with Zn fertilizer treatment had the lowest PA:Zn of 43.3 (red clay) and
470 44.0 (sandy soil) during the 2nd cropping season (Table 6). Conversely, the highest estimated
471 PA:Zn ratios were observed in crops fertilized with solely mineral N and P. Despite decreased
472 PA:Zn with Zn fertilization, the resultant ratios were still well-above the ratio of 15-20
473 considered appropriate for gut absorption of Zn in humans.

474 **3.5 The potential contribution of Zn fertilization of cowpea to household Zn supply**

475 On the clay soil, potential dietary Zn supply ranged from 2.9-4.0 mg person⁻¹ day⁻¹ and 3.6-4.2
476 mg person⁻¹ day⁻¹ during the 1st and 2nd cropping seasons, respectively (Tables 6 and 7), based on
477 a 100 g intake of cowpea person⁻¹ day⁻¹. On the sandy soil, dietary Zn supply ranged from 1.9-3.0
478 mg person⁻¹ day⁻¹ and 3.1-4.5 mg person⁻¹ day⁻¹ during the 1st and 2nd cropping seasons,
479 respectively. The use of Zn fertilizer had a greater effect under the sole mineral N and P and the
480 woodland leaf litter treatments than cattle manure. Thus, the greatest increase in dietary Zn
481 supply on sandy soils was from 3.2 to 4.5 mg person⁻¹ day⁻¹ when Zn was applied to the
482 woodland leaf litter treatment in season 2. This result was comparable to an increase in dietary Zn
483 supply of 1.3 mg person⁻¹ day⁻¹ when Zn was applied under the sole mineral N and P treatment.

484 In isolation, Zn contributed 42% of this increase and woodland leaf litter contributed 3% of the
485 increase.

486 **4. DISCUSSION**

487 **4.1 Influence of current farmer soil fertility management on cowpea grain yields and** 488 **nutrition**

489 Despite research efforts to promote the use of ISFM in legume production systems in Zimbabwe
490 (Kanonge et al., 2015; Mtambanengwe and Mapfumo, 2009), a large percentage of farmers in
491 this study were found to grow grain legumes without any (56%) or sub-optimal (33%) forms of
492 mineral or organic nutrient resources. This is consistent with Kanonge et al. (2015) who reported
493 evidence of poor adoption/use of ISFM in cowpea production from a survey of more than 70
494 farms in the eastern region of Zimbabwe. Higher rates of fertilization on legumes are typically
495 used only by the resource-endowed farmers (Kanonge et al., 2015). Smallholder farmers in
496 Zimbabwe fall into resource endowment categories as dictated by farm-level physical resources,
497 access to crop production inputs, among other criteria, which, in turn, influence their nutrient
498 resource allocation patterns to different fields (Mtambanengwe and Mapfumo, 2005). This
499 current study therefore provides evidence that improved nutrient resource allocation efficiencies
500 by farmers can directly increase dietary Zn supply. For example, application of organic nutrient
501 resources of up to 6.0 t ha⁻¹ resulted in the highest grain Zn concentrations of 30.1 mg kg⁻¹,
502 potentially supplying 22% of the recommended adult Zn intake of 14 mg person day⁻¹. Similar
503 findings were reported in the Sahel where wide variations in macro- and micronutrients were
504 reported in grains of millets grown under farmer's diverse short- to long-term ISFM and inherent
505 soil nutrient status (Buerkert et al., 1998).

506 **4.2 Importance of Zn, mineral and organic fertilization in cowpea establishment and**
507 **productivity**

508 Zinc fertilizer applications in combination with mineral and organic fertilizers enhanced
509 establishment (i.e. biomass production) of cowpea grown on the sandy soil. This effect was more
510 apparent on the sandy soil compared to the red clay soil. Differences in response to Zn fertilizer
511 between the two soil types could be attributed to soil chemical properties which affects soil Zn
512 availability. While increased soil Zn availability is expected in soils with higher organic matter
513 and clay content (Rengel, 2002; Alloway, 2009), absence of apparent Zn benefits on grain yield
514 on the clay soil could be due to a high initial plant available soil Zn of 1.7 mg kg⁻¹ which could
515 have potentially masked any significant yield responses to Zn fertilizer (Solheim and Solheim,
516 2010). An increase in cowpea germination (data not shown) and shoot biomass yield with
517 application of Zn has been reported earlier (Fawzi et al., 1993; Johnson et al., 2005). This
518 improved cowpea shoot biomass productivity in this study can partly be attributed to Zn
519 fertilization which promotes crop growth and yield through increased auxin production (Alloway,
520 2008; Poblaciones and Rengel, 2016). Given that cowpea leaves are an important source of relish
521 in smallholder farming systems in Zimbabwe, and given leaves of grain legumes have the
522 capacity to accumulate more Zn compared to grains (Broadley et al., 2012), this source of dietary
523 Zn could support improved Zn nutrition among smallholder farms. Furthermore, the high
524 biomass could contribute to residual macro- and micro-nutrients accumulation in the soil upon
525 decomposition of the plant residues (Adjei-Nsiah et al., 2008; Kanonge et al. 2015; McLaughlin
526 et al., 1988). This can reduce mineral fertilizer, especially N input, for rotational cereal crops
527 such as maize (Nezomba et al., 2015).

528 In the current study, application of Zn fertilizers had up to 10% added grain yield benefit on both
529 farmers' fields and experimental sites. This is consistent with yield increases of many other
530 crops, including wheat, rice and maize (Cakmak et al., 2010; Harris et al., 2007; Manzeke et al.,
531 2014; Ram et al., 2016; Shivay et al., 2015). Improved crop productivity with Zn fertilization
532 allows farmers to realize improved food and nutrition intake and also realize soil fertility benefits
533 from biomass accumulation. In this study, the survey conducted at the same sites but not using Zn
534 fertilizers showed that cowpea grain yields of $<1 \text{ t ha}^{-1}$ could be achieved following the addition
535 of organic nutrient resources and mineral N and P fertilizer. Given these yields were substantially
536 lower than achieved with Zn fertilization, it is therefore apparent that current ISFM techniques
537 employed by smallholder farmers lack essential micronutrients required for optimal cowpea
538 productivity.

539 Under similar climatic conditions and soil type (sandy soil), previous work conducted at the same
540 district showed $\sim 0.3 \text{ t ha}^{-1}$ lower cowpea grain yields in selected treatments than the current study
541 (Table 7) even when higher rates (6.5 t ha^{-1}) of organic nutrient resources were applied. For
542 example, cowpea grain yields of 1.7 and 1.8 t ha^{-1} were obtained when 6.5 t ha^{-1} of cattle manure
543 and woodland leaf litter were applied compared to a current yield of 2.0 t ha^{-1} attained when Zn
544 was applied in combination with a lower rate (5 t ha^{-1}) of cattle manure. Assuming similar
545 agronomic management practices, we could attribute the increases in grain yield of up to 10% to
546 Zn fertilization (Table 7).

547

548 While optimal yield benefits were obtained with inorganic Zn fertilizers, application of high
549 quantities of organic nutrient resources in combination with mineral N and P fertilizer, without
550 Zn, can still increase cowpea grain yields (see Table 7; Kanonge et al., 2015). The use of cattle

551 manure and woodland leaf litter has previously been found to give grain yield benefits due to
552 their capacity to supply both Zn (Manzeke et al., 2012) and other nutrients (Giller, 2001) which
553 are essential for legume productivity. This was also evident under field experiments on the sand
554 soil type where cowpea grain yields increased up to 2 t ha⁻¹ when 5 t cattle manure ha⁻¹ was
555 applied in combination with Zn during the 1st season compared to yields of ~1 t ha⁻¹ when Zn was
556 applied without cattle manure (see Figure 2b). Significant increases in cowpea grain yields with
557 cattle manure use could be attributed to organic N supply which enhanced Zn availability, uptake
558 and translocation in the plant as reported earlier in wheat (Kutman et al., 2010; 2011). However,
559 it is unlikely that most smallholder farmers could afford to apply such high levels of organic
560 nutrient resources to grain legumes (Mtambanengwe and Mapfumo, 2009). This lack of capacity
561 to apply large quantities of organic nutrient resources to grain legumes calls for the inclusion of
562 Zn-based fertilizers, and possibly other micronutrients, in the ISFM packages currently being
563 promoted on smallholder farms. There is however, a need to balance N application rates to grain
564 legumes. For example, apparently lower cowpea grain yields attained on the sandy soil during the
565 2nd season with residual organic N (10 t ha⁻¹) and mineral N application compared to application
566 of 5 t cattle manure ha⁻¹ could be attributed to nitrate intolerance in selected grain legumes which
567 could have depressed BNF and crop productivity (Fujita et al., 1992).

568 **4.3 Zinc fertilizer importance in cowpea grain nutrition**

569 Zinc fertilizer application increased grain Zn concentrations of cowpea grown on contrasting
570 soils and treatment combinations, showing its potential to contribute to both crop and human
571 nutrition across variable soils. When cowpea followed the maize crop during the 2nd year of
572 cropping, higher grain Zn concentrations were reported than concentrations attained with direct
573 cowpea fertilization providing evidence of the beneficial effects of legume-cereal rotational

574 systems and possibly increased soil Zn availability and plant uptake within subsequent years of
575 cropping (Manzeke et al., 2014; Wang et al., 2012) . Cowpea is likely to have benefited from the
576 residual fertility from the rotational maize crop which was grown with higher quantities of
577 mineral fertilizers and organic nutrients, as currently practised by smallholder farmers. Maize
578 grain Zn concentrations of up to 35 mg kg⁻¹ were attained with Zn fertilization (data not shown).
579 Maize grain Zn concentrations of up to 39 mg kg⁻¹ were previously obtained in Zimbabwe
580 following use of Zn-based fertilizers and organic nutrient resources (Manzeke et al., 2014), from
581 a baseline of ~15 to 21 mg Zn kg⁻¹ found under sole mineral N and P fertilizer treatments. A
582 higher grain Zn concentration has been reported earlier in cowpea and other grain legumes
583 (Pandey et al., 2013; Poblaciones and Rengel, 2016) compared with maize (Manzeke et al., 2014)
584 and wheat (Gomez-Coronado et al., 2016) grown under similar conditions. These findings show
585 that grain legumes are likely to accumulate more Zn than cereals due to their higher protein
586 content and association between Zn and proteins particularly in the embryo and aleurone of
587 grains (Cakmak, 2000; Kutman et al., 2010). Potentially, more efficient remobilization of Zn
588 from leaves to grains in legumes compared to cereals (White and Broadley, 2009) could also
589 explain the higher grain Zn concentrations in legumes. Other agronomic biofortification methods
590 to increase grain Zn concentration in legumes include pre-soaking of grain legumes and
591 application of foliar sprays (Abdel-Ghaffar, 1988; Cakmak and Kutman, 2017; Ram et al., 2016;
592 Weldu et al., 2012). Using combined approaches and a higher Zn fertilizer rate than one used in
593 this study, it may be possible to meet a target of between 49 and 61 mg kg⁻¹, which are the
594 current targets in field beans and peas, respectively (Bouis and Welch, 2010; Huett et al., 1997).
595 There is therefore a need for constant soils tests to avoid Zn accumulation and probable toxicity
596 effects both in the soil and plants which may be associated with application of higher Zn
597 fertilizers.

598 This study clearly shows the value of promoting ISFM to improve grain Zn concentration.
599 However, the benefits of increased Zn supply can be impeded by high levels of PA, the main
600 storage form of P in legume grains. The dietary PA supply in Zimbabwe is high (2820-3430 mg
601 person⁻¹ d⁻¹) with 17 and 68% being supplied by legumes and cereals, respectively (Joy et al.,
602 2014; Kumssa et al., 2015). In this study, grain P concentration ranged from 2.6-3.3 g kg⁻¹ with a
603 tendency of low P in grain of cowpea grown with Zn, low P (14 kg ha⁻¹) and organic nutrient
604 resources (5 t ha⁻¹). Increase in phytate in legumes such as soyabean (*Glycine max* [L.] Merr.) and
605 other field crops including pearl millet (*Pennisetum glaucum* L.) under high P fertilizer
606 application has previously been reported (Buerkert et al., 1998; Raboy and Dickinson, 1983).
607 Therefore, as for maize on similar soils (Manzeke et al., 2012), using a 65% grain P conversion
608 ratio (O'Dell et al., 1972; Wu et al., 2009) to estimate PA in cowpea grain, our results indicate
609 that P fertilizers could increase grain PA:Zn molar ratio in legumes which potentially inhibit Zn
610 absorption in the human gut (Cakmak, 2008).

611 In this study, high grain P concentration translated to high PA:Zn molar ratios of up to 71.2
612 which were reduced to 44 (sandy soil) with Zn fertilization. PA:Zn molar ratios were generally
613 lower on the more fertile red clay soils suggesting a potential influence of soil type and farmer
614 nutrient management on phytate accumulation. Soils with high clay and organic matter content
615 have greater P retention and fixing capacity compared to soils with a lower clay content rendering
616 the nutrient less available for plant uptake (Lalljee, 1997; Morel et al., 1989). Clearly, appropriate
617 P management of legume/cereal-based cropping systems is critical to balance the requirements
618 for crop growth with the potential inhibitory effects on Zn availability in human nutrition.

619 **4.4 Potential benefits of Zn fertilizer to dietary Zn intake**

620 A Zn intake of 14 mg person⁻¹ day⁻¹ is required to meet dietary Zn requirement for an adult reliant
621 on a typically low Zn bioavailability diet (WHO/FAO, 2004). Using this recommended Zn intake
622 and assuming the consumption of 100 g cowpea per day, an equivalent of about 32% of the daily
623 adult Zn intake was supplied under the best soil management strategy in this study. Application
624 of organic nutrients alone to cowpea supplied only 16% of an adult's daily Zn requirement.
625 Based on a low Zn bioavailability diet, this potential Zn supply with Zn fertilization could be
626 even higher for infants and children whose daily Zn intakes are lower. Using an optimistic daily
627 cowpea intake of 100 g for infants and children, 68% and 40% of daily requirements could be
628 supplied to meet their recommended Zn intake of 6.6 and 11.2 mg person⁻¹ day⁻¹, respectively
629 (WHO/FAO, 2004). These assumptions on the nutritional relevance of Zn fertilizer to human
630 daily Zn intake do not, however, take into consideration the potential loss of Zn at milling,
631 inhibitory effects of PA and an estimate of Zn loss at cooking. Zinc loss during cooking was
632 considered negligible under the current cooking methods (Pereira et al., 2014). There is clear
633 scope for promoting Zn fertilizer use to potentially meet the household Zn nutrition of vulnerable
634 groups practicing legume-cereal cropping systems under variable soils.

635 **4.5 Soil type is important when considering agronomic biofortification interventions**

636 Application of Zn fertilizers to cowpea resulted in added grain yield and grain Zn benefits on
637 both soil types, despite marginal increases in yield and grain Zn concentration on the red clay
638 soil. Sandy soils were proportionally more responsive to Zn fertilizers and organic nutrients than
639 red clay soil where insignificant treatment differences in grain yield and grain Zn concentration
640 were reported. Differences in cowpea response to Zn fertilization are likely to be due to
641 differences in soil chemical properties of the two soil types. For example, Zn adsorption increases
642 under high clay content and high pH (Alloway, 2008; Hippler et al., 2015). Tagwira (1991)

643 reported a decrease in MgCl_2 extractable Zn with an increase in clay content in similar
644 Zimbabwean soils. The lower specific metal adsorption capacity on sandy soils results in
645 increased plant-availability, and therefore Zn fertilizer use efficiency, than on clay soils. Greater
646 Zn fertilizer response has been reported in citrus trees grown on a sandy loam soil compared to
647 trees grown on a clay soil (Hippler et al., 2015). In addition, Solheim and Solheim (2010) also
648 reported higher maize crop responses on a site with $\leq 0.5 \text{ mg kg}^{-1}$ plant available Zn compared to
649 a site with $> 1.3 \text{ mg Zn kg}^{-1}$. Based on our findings, Zn fertilizer use efficiency is depended on
650 soil type and geochemistry, which needs to be considered in agronomic biofortification programs.
651 The potential influence of spatial variation in soil type on maize grain micronutrient
652 concentrations and dietary supply has also been reported in Malawian soils (Chilimba et al.,
653 2012; Hurst et al., 2013; Joy et al., 2015b) and other African countries (Sanginga and Woomer,
654 2009). An improved understanding of soil geochemistry on spatial distribution of micronutrients
655 is therefore important for appropriate and efficient nutrient management on regions and farms
656 which vary in nutrient input requirement for sustainable agriculture and public health
657 interventions.

658 **4.6 Benefit of Zn fertilizer in legume-cereal cropping: A smallholder systems perspective**

659 Our findings show benefits of Zn in legume cropping and how beneficial it is for smallholder
660 farmers to use Zn-based fertilizers, and possibly other nutrients, in the dominant legume-cereal
661 cropping systems to enhance food and nutrition security in the face of stress factors such as poor
662 soil fertility and climate change. With recently reported increased changes in rainfall distribution
663 under rain-fed agriculture (Rurinda et al., 2013), enrichment of cowpea with Zn fertilizer and
664 other drought tolerant grain legume crops, often grown in rotation with staple cereals, becomes
665 imperative. Apart from its capacity to fix N in the natural environment, cowpea closely

666 accompanies maize in smallholder cropping and responds well to fertilization. Benefits of Zn
667 fertilizer use on crop productivity and nutrition were apparent in the legume-cereal rotational
668 system, particularly in the legume phase. This concurs with our earlier findings (Kanonge et al.,
669 2015). Higher grain Zn concentration of maize following cowpea (data not shown) in the 2nd
670 season compared to grain Zn concentration attained with direct fertilization of maize implies
671 legume-cereal rotations are a two-way system which complements each other regardless of
672 initially fertilizing the legume or maize. Our current findings show a dimension of enhancing
673 nutritional value of the maize/legume systems which could be employed in soil geochemistry
674 applications.

675 **5. CONCLUSIONS**

676 Low dietary Zn intakes remain prevalent in typical legume-cereal-based diets of smallholder
677 communities in SSA. In this study, we show the potential benefits of combining ISFM practices
678 currently being employed by farmers on cowpea production with Zn fertilizers to increase dietary
679 Zn intake especially on sandy soils. Zinc fertilizer use under ISFM significantly improved crop
680 productivity and grain quality of cowpea grown under a legume-maize rotational sequence on
681 contrasting soil types with a proportionally more response to Zn fertilizers and organic matter on
682 sandy soils than on red clay soils. The resultant increase in crop productivity and grain nutritional
683 value of cowpea grown with Zn fertilizer and ISFM could potentially satisfy daily Zn intake of
684 resource poor communities who are likely to face challenges of diversifying their diets. In this
685 regard, agronomic biofortification of grain legumes with external sources of Zn is feasible and
686 significantly contribute towards increasing dietary Zn intake. There is however a need for future
687 work to focus on balances of P and Zn fertilization of grain legumes to offset possible effects of
688 dietary PA emanating from increased P fertilizer use and PA:Zn molar ratios in legumes. The

689 variability in available soil micronutrient status and differences in response to fertilizer
690 application suggest scope for appropriate micronutrient fertilizer use on different soil types.

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982

983 **Tables**

984 **Table 1:** Soil characteristics (0-20 cm) of the selected field sites for experiments established in
 985 eastern Zimbabwe.

986

Property	Sandy soil	Red clay soil
Sand (%)	90 (0.5)	30 (3.0)
Clay (%)	6.0 (1.5)	57 (2.0)
Soil texture	Sandy soil	Clay soil
^a Loss on ignition (LOI-%)	1.18 (0.2)	6.0 (1.5)
pH (0.01 M CaCl ₂)	4.46 (0.2)	4.5 (0.2)
Total Zn (mg kg ⁻¹)	8.00 (1.1)	145 (14.6)
EDTA available Zn (mg kg ⁻¹)	0.98 (0.1)	1.7 (0.1)
Total P (mg kg ⁻¹)	80 (6.2)	389 (15.3)
Total N (%)	0.03 (0.02)	0.1 (0.03)
Available P (mg kg ⁻¹)	4.0 (0.2)	8.5 (0.5)
^b Mineral N (mg kg ⁻¹)	18 (1.4)	29 (2.1)
Exchangeable Ca (cmol _c kg ⁻¹)	0.9 (0.1)	2.6 (0.5)
Exchangeable Mg (cmol _c kg ⁻¹)	0.6 (0.2)	1.8 (0.1)
Exchangeable K (cmol _c kg ⁻¹)	0.2 (0.1)	0.6 (0.3)

987 ^aLOI was measured as a proxy for soil organic carbon; ^bMineralizable N after two weeks of anaerobic incubation.
 988 Values in parentheses denote standard deviation (SD).

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991

992 **Table 2:** Treatments used to determine the influence of mineral and organic fertilizer application and rates on cowpea productivity and grain
 993 Zn nutritional value grown in rotation with maize.

Fertilizer option	Treatment	Year 1 (2014/15)		§Year 2 (2015/16)		Total mineral fertilizer added (kg ha ⁻¹)		
		Fertilizer rate (ha ⁻¹)	Crop	Fertilizer rate (ha ⁻¹)	Crop	N	P	†Zn
Mineral fertilizer	1	90 kg N + 26 kg P (Control)	Maize	30 N + 26 kg P	Cowpea	120	52	0
	2	90 kg N + 26 kg P + 5 kg Zn	Maize	30 N + 26 kg P	Cowpea	120	52	5
	3	30 kg N + 26 kg P (Control)	Cowpea	90 kg N + 26 kg P	Maize	120	52	0
	4	30 kg N + 26 kg P + 5 kg Zn	Cowpea	90 kg N + 26 kg P	Maize	120	52	5
Combinations of mineral and organic nutrient resources	5	5 t cattle manure + 30 kg N + 26 kg P + 5 kg Zn	Cowpea	90 kg N + 26 kg P	Maize	120	52	5 (148)
	6	5 t cattle manure + 16 kg N + 14 kg P + 5 kg Zn	Cowpea	30 kg N + 14 kg P	Maize	46	28	5 (148)
	7	10 t cattle manure + 90 kg N + 26 kg P	Maize	30 kg N + 26 kg P	Cowpea	120	52	0 (296)
	8	10 t cattle manure + 90 kg N + 26 kg P + 5 kg Zn	Maize	30 kg N + 26 kg P	Cowpea	120	52	5 (296)
	9	10 t woodland leaf litter + 90 kg N + 26 kg P	Maize	30 kg N + 26 kg P	Cowpea	120	52	0 (798)
	10	10 t woodland leaf litter + 90 kg N + 26 kg P + 5 kg Zn	Maize	30 kg N + 26 kg P	Cowpea	120	52	5 (798)

994 § implies residual organic cattle manure, woodland leaf litter and Zn fertility in treatments with the respective fertilizers in the preceding maize crop. †Denotes
 995 elemental Zn. Plots receiving 26 and 14 kg P ha⁻¹ also received 24.5 kg K ha⁻¹ and 13.2 kg K ha⁻¹ respectively, as K₂O from basal compound D
 996 fertilizer. Figures in parentheses denotes amount of elemental Zn (g ha⁻¹) supplied by either 5 or 10 t organic nutrient resource ha⁻¹.

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1003 **Table 3:** Description of fertilization options and fertilizer rates employed in cowpea production by selected farmers during the crop
 1004 survey conducted in Hwedza District, Zimbabwe.

Management option	Range of fertilizer rates applied	Proportion of farms employing each management option (%)	Description
Unfertilized control	None	56 (33)	No form of mineral N and P and/ or organic fertilizer applied
Mineral NPK only	3.5-30 kg N ha ⁻¹ and 0.3-26 kg P ha ⁻¹	33 (20)	Mineral N applied as basal fertilizer at planting as Compound D (7N:14P ₂ O ₅ :7K ₂ O)
Organics only	1.0-6.0 t dry matter ha ⁻¹	3 (2)	Applied organic nutrient resources included mostly cattle manure and compost with a few farmers applying woodland leaf litter to cowpea. These organic nutrient resources are usually available on-farm and are heaped and spread on fields during the dry months of October before the onset of rains.
Organics + mineral NPK fertilizer	1.0-6.0 t dry matter ha ⁻¹ + 3.5-30 kg N ha ⁻¹ and 0.3-26 kg P ha ⁻¹	8 (5)	The ISFM option encompasses combined application of organic nutrient resources (usually compost, ash, woodland leaf litter and cattle manure) and mineral N and P fertilizer as basal Compound D application. Organic resources are spread before on-set of rains and mineral fertilizer are applied at planting.

1005 Figure in parentheses denotes the total number of farms within each soil fertility management option.

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1009 **Table 4:** Cowpea grain yields and nutritional value under different soil fertility management options on farmers's fields in Hwedza
 1010 district, eastern Zimbabwe.

Treatment	Grain yield	Range	Grain Zn concentration		Grain Zn uptake	Range
	kg ha ⁻¹	kg ha ⁻¹	mg kg ⁻¹	mg kg ⁻¹	g ha ⁻¹	g ha ⁻¹
Unfertilized control (N = 33)	287 (194) a	40-600	23.9 (2.6) a	19.0-26.4	6.8 (4.6) a	2.2-15.9
Mineral NPK only (N = 20)	566 (189) b	200-800	24.4 (3.2) a	19.2-27.9	13.8 (5.0) b	5.1-21.9
Organics only (N = 2)	683 (353) b	350-850	27.7 (3.7) b	25.0-30.3	18.9 (12.0) c	8.8-25.8
Organics + mineral NPK fertilizer (N = 5)	895 (307) c	400-1000	30.1 (1.5) c	27.9-31.4	26.9 (9.5) d	12.6-30.7
Mean		608		26.5		16.6
SED		169		1.3		4.7
CV (%)		42.4		6.9		44.8
F test		*		**		**

1011 ** significant at P<0.01; * significant at P<0.05; Figures in parentheses denote standard deviation (SD).

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1016 **Table 5:** Cowpea grain nutritional value under different treatments on a sandy and red clay soil during the 1st cropping season in
 1017 Hwedza, eastern Zimbabwe.

a) Sandy soil

Treatment	Grain Zn (mg kg ⁻¹)	Grain P (g kg ⁻¹)	PA:Zn	Grain Zn uptake (g ha ⁻¹)	†Potential dietary Zn supply mg person ⁻¹ day ⁻¹
30 kg N + 26 kg P	18.5 (0.9) a	3.2 (0.2)	112.4 (4.9) c	14.8 (1.6) a	1.9 (0.10)
30 kg N + 26 kg P + 5 kg Zn	24.8 (0.3) b	2.7 (0.2)	70.8 (1.1) b	23.9 (0.8) b	2.5 (0.03)
5 t cattle manure + 30 kg N + 26 kg P + 5 kg Zn	26.2 (0.4) b	1.9 (0.06)	47.1 (1.0) a	53.1 (1.0) c	2.6 (0.04)
5 t cattle manure + 16 kg N + 14 kg P + 5 kg Zn	30.2 (0.7) c	2.8 (0.2)	60.3 (0.6) b	27.8 (0.3) b	3.0 (0.07)
Mean	24.9	2.7	72.7	29.9	2.5
SED	1.7	0.7	10.8	9.9	n/a
CV (%)	9.7	3.1	6.1	10.6	n/a
F test	**	ns	*	*	n/a

b) Red clay soil

Treatment	Grain Zn (mg kg ⁻¹)	Grain P (g kg ⁻¹)	PA:Zn	Grain Zn uptake (g ha ⁻¹)	†Potential dietary Zn supply mg person ⁻¹ day ⁻¹
30 kg N + 26 kg P	29.2 (0.7)	3.8 (0.2)	84.6 (1.0)	30.9 (1.0)	2.9 (0.07)
30 kg N + 26 kg P + 5 kg Zn	33.1 (1.9)	3.0 (0.3)	58.9 (2.1)	43.3 (1.1)	3.3 (0.19)
5 t cattle manure + 30 kg N + 26 kg P + 5 kg Zn	37.4 (2.1)	3.4 (0.2)	59.1 (6.4)	68.0 (3.5)	3.7 (0.21)
5 t cattle manure + 16 kg N + 14 kg P + 5 kg Zn	40.2 (0.8)	3.1 (0.2)	50.1 (1.9)	70.3 (4.6)	4.0 (0.08)
Mean	35.0	3.3	63.1	53.1	3.5
SED	4.2	0.5	8.9	19.7	n/a
CV (%)	3.8	2.9	4.5	2.5	n/a
F test	ns	ns	ns	ns	n/a

1018 † Potential Zn supply against a recommended adult intake of 14 mg person⁻¹ day⁻¹ after consumption of 100g boiled cowpea (does not account for preparation and cooking losses
 1019 and PA:Zn). Means followed by the same letter are not significantly different. ** significant at P<0.01; * significant at P<0.05; ns-not significantly different. n/a – not applicable.
 1020 Figures in parentheses denote standard deviation.
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1024 **Table 6:** Grain Zn content measured in grain collected from experimental sites during the 2nd cropping season in Hwedza, eastern
 1025 Zimbabwe.

a) Sandy soil

Treatment	Grain Zn (mg kg⁻¹)	Grain P (g kg⁻¹)	PA:Zn	Grain Zn uptake (g ha⁻¹)	†Potential dietary Zn supply mg person⁻¹ day⁻¹
30 kg N + 26 kg P	31.1 (7.0) a	3.3 (0.3)	71.2 (16.0) d	34.2 (2.3) a	3.1 (0.7)
30 kg N + 26 kg P + *5 kg Zn	43.6 (2.0) c	3.0 (0.2)	44.0 (2.3) a	56.7 (4.2) c	4.4 (0.2)
*10 t cattle manure + 30 kg N + 26 kg P	31.9 (3.9) a	3.1 (0.01)	63.8 (6.0) c	40.8 (3.9) b	3.2 (0.4)
*10 t cattle manure + 30 kg N + 26 kg P + *5 kg Zn	38.6 (2.4) b	3.3 (0.09)	55.9 (3.9) b	52.1 (4.4) c	3.9 (0.2)
*10 t woodland leaf litter + 30 kg N + 26 kg P	32.2 (2.8) a	3.1 (0.11)	63.6 (5.5) c	39.9 (5.1) b	3.2 (0.3)
*10 t woodland leaf litter + 30 kg N + 26 kg P + *5 kg Zn	44.7 (4.9) c	3.3 (0.4)	47.8 (1.4) a	64.7 (4.0) d	4.5 (0.5)
Mean	37.0	3.2	57.7	48.0	3.7
SED	2.9	0.2	6.3	5.3	n/a
CV (%)	7.1	3.6	5.0	8.3	n/a
F test	**	ns	**	**	n/a

b) Red clay soil

Parameter	Grain Zn (mg kg⁻¹)	Grain P (g kg⁻¹)	PA:Zn	Grain Zn uptake (g ha⁻¹)	†Potential dietary Zn supply mg person⁻¹ day⁻¹
30 kg N + 26 kg P	35.9 (6.8)	3.1 (0.2)	51.2 (5.4)	57.4 (6.1)	3.6 (0.7)
30 kg N + 26 kg P + *5 kg Zn	41.6 (0.7)	2.8 (0.3)	43.3 (5.6)	77.8 (4.9)	4.2 (0.1)
*10 t cattle manure + 30 kg N + 26 kg P	38.8 (2.9)	2.7 (0.3)	45.3 (3.9)	66.0 (5.3)	3.9 (0.3)
*10 t cattle manure + 30 kg N + 26 kg P + *5 kg Zn	41.3 (2.3)	2.8 (0.3)	44.4 (6.7)	78.2 (4.2)	4.1 (0.2)
*10 t woodland leaf litter + 30 kg N + 26 kg P	39.2 (1.8)	2.6 (0.7)	47.0 (4.0)	66.6 (6.0)	3.9 (0.2)
*10 t woodland leaf litter + 30 kg N + 26 kg P + *5 kg Zn	42.1 (4.1)	3.0 (0.5)	46.0 (4.1)	77.3 (4.2)	4.2 (0.4)
Mean	39.8	2.8	46.2	70.6	4.0
SED	3.1	0.3	3.9	11.2	n/a
CV (%)	2.7	6.9	5.5	9.6	n/a
F test	ns	ns	ns	ns	n/a

1026 * indicate residual fertility from cattle manure, woodland leaf litter and Zn applied to the preceeding maize crop. † Potential Zn supply against recommended intake of 14 mg
 1027 person⁻¹ day⁻¹ after consumption of 100g boiled cowpea (does not account for preparation and cooking losses and PA:Zn). ** significant at P <0.01. Means followed by same letters
 1028 did not differ significantly at P<0.05. n/a – not applicable. Figures in parentheses denote standard deviation (SD).

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1031 **Table 7:** A comparison of influence of zinc (Zn) fertilization with other ISFM treatments without Zn on cowpea productivity on sandy
 1032 soils in Zimbabwe.

Treatments	Biomass yield (t ha ⁻¹)	Zn added biomass yield benefit (%)	Grain yield (t ha ⁻¹)	Zn added grain yield benefit (%)	Field site	Sources of data
a) Mineral fertilizer comparison						
†30 kg N + 26 kg P + Zn	4.0	n/a	0.96	n/a	On-farm	Kanonge et al. (2015)
26 kg P ha ⁻¹ (Basal PKS only)	1.9	111	0.9	8.9	On-farm	Kanonge et al. (2015)
26 kg P ha ⁻¹ + 30 kg N	n/s	n/a	0.5	96	On-station	ABACO, 2015 (unpublished data)
14 kg P ha ⁻¹ + 8 kg N	n/s	n/a	0.4	145	On-station	ABACO, 2015 (unpublished data)
Mineral NPK	n/s	n/a	0.6	60	Farmers' fields	Cowpea crop survey
Unfertilized control	1.4	236	0.5	49	On-farm	Kanonge et al. (2015)
*Mean	1.7	174	0.6	72		
b) Mineral + organic fertilizer comparison						
†5.0 t cattle manure + 30 kg N + 26 kg P + Zn	4.7	n/a	2.0	n/a	On-farm	Kanonge et al. (2015)
6.5 t cattle manure + PKS	2.6	81	1.7	16.5	On-farm	Kanonge et al. (2015)
6.5 t woodland leaf litter + PKS	2.3	104	1.8	10	On-farm	Kanonge et al. (2015)
6.5 t cattle manure + NPK	3.2	47	2.2	-10	On-farm	Kanonge et al. (2015)
6.5 t woodland leaf litter + NPK	2.5	88	2.1	-5.7	On-farm	Kanonge et al. (2015)
Organics + mineral NPK	n/s	n/a	0.9	122	Farmers' fields	Cowpea crop survey
*Mean	2.7	80.0	1.7	26.6		

1033 n/a implies not applicable; n/s implies not sampled. †= 1st season sandy soil site treatment used to calculate Zn fertilization benefits on cowpea yield.* = mean excluding the Zn
 1034 treatment.

LEGENDS TO FIGURES

Fig. 1. Cumulative rainfall received in Hwedza, Zimbabwe during the 2014-15 and 2015-16 cropping seasons.

Fig. 2. Cowpea grain yields under different soil fertility management options and Zn fertilization on a sandy and red clay soil during the 1st and 2nd cropping seasons. Vertical bars accompanied by the same letter are not significantly different at $P < 0.05$. Astericks indicate residual fertility from cattle manure, woodland leaf litter and Zn applied to the preceding maize crop.

Fig. 3. Cowpea biomass productivity at peak flowering on a sandy and red clay soil in year 1 (2014-15) and year 2 (2015-16). Vertical bars accompanied by the same letter are not significantly different at $P < 0.05$. Astericks indicate residual fertility from cattle manure, woodland leaf litter and Zn applied to the preceding maize crop.

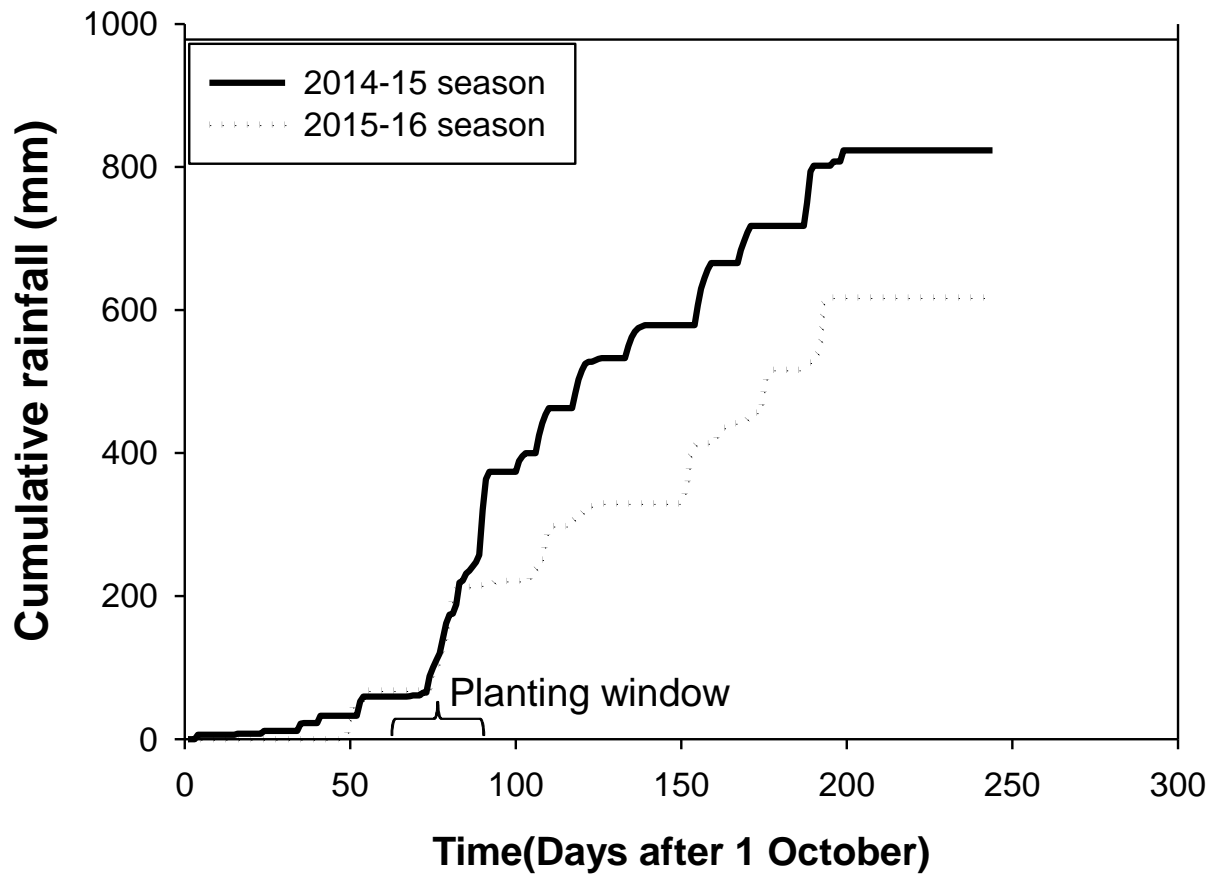
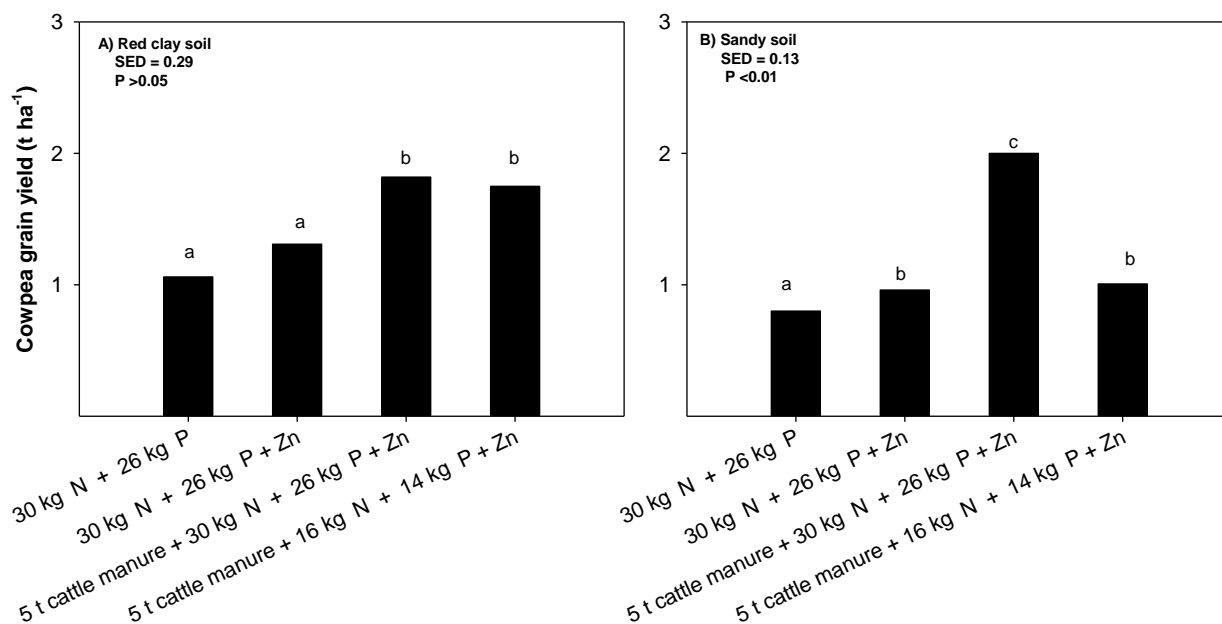
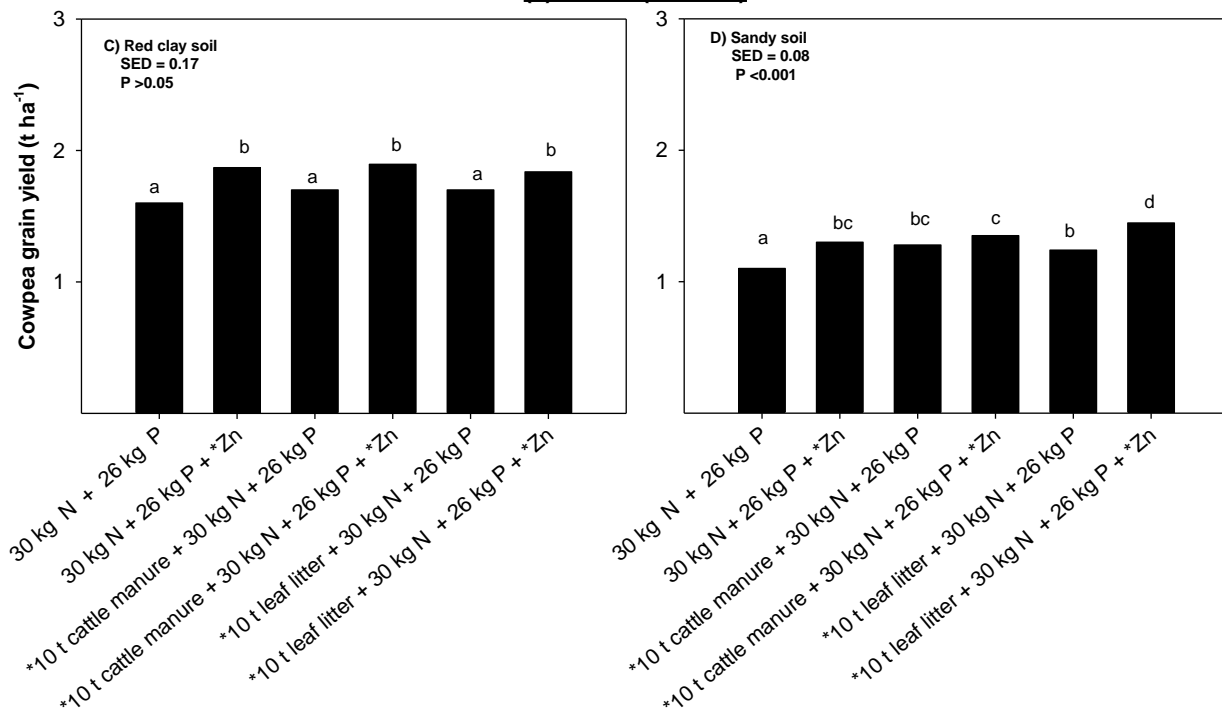


Fig. 1.

(i) Year 1 (2014-15)



(ii) Year 2 (2015-16)

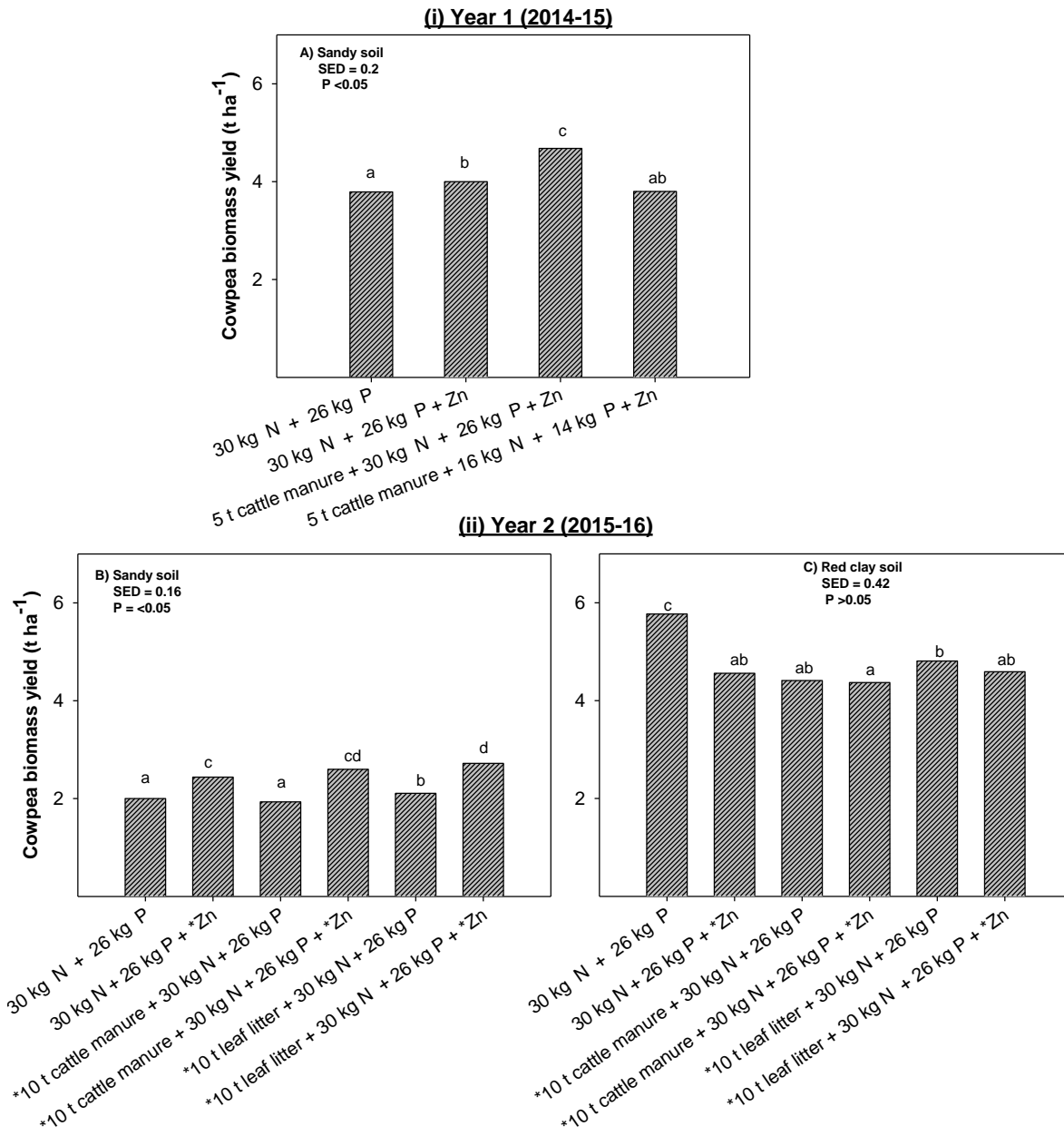


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Fig. 3.