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

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ABSTRACT

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

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

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

1. INTRODUCTION

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

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

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

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

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

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

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

2. MATERIALS AND METHODS

The study was conducted in Hwedza District (18° 41' S, 31° 42' E) in Eastern Zimbabwe. It comprised a survey of 60 farmers in 2014/15, and field experiments at two sites in 2014/15 and 2015/16 cropping seasons. The study builds on the Soil Fertility Consortium for Southern Africa (SOFECSA)'s work on legume production in smallholder farming communities under diverse ISFM techniques that included systematic legume-cereal rotations, crop diversification and combined use of mineral and organic nutrient resources. SOFECSA had been working with smallholder farmers in Hwedza since 2005. Hwedza encompasses three of Zimbabwe's agro-ecological region/natural regions (NR) IIb to IV, receiving 450-800 mm $year^{-1}$ between November and March. Soils in this community are broadly classified as Lixisols (FAO/ISRI/ISSS, 2006). Maize is the dominant crop under a mixed crop-livestock farming system (Mtambanengwe and Mapfumo, 2009). Legumes such as groundnut (*Arachis hypogea* L.), cowpea and common bean (*Phaseolus vulgaris* L.) are typically grown on smaller patches of land compared with the staple maize (Rusinamhodzi et al., 2006), often with minimal or zero fertilization (Kanonge et al., 2015) resulting in inefficient legume-cereal rotational systems. Cattle are the dominant livestock mainly kept for manure and draught power provision. In the absence of cattle manure, farmers often collect woodland leaf litter from the tropical savanna woodlands for soil fertility management. Rainfall in Hwedza is often uneven (Rurinda et al., 2013), for example, the district received >800 mm $annum^{-1}$ in the 2014/15 cropping season, with 314 mm obtained within the month of December 2014 alone (Figure 1).

2.1 Survey

A survey was conducted in Dendenyore (agro-ecological zone IIb) and Ushe (agro-ecological zone III-IV) Wards in Hwedza to determine the range of soil fertility management options employed under cowpea production and to quantify grain yields and Zn nutritional composition. The survey targeted households working with SOFECSA on cowpea production, and other grain legumes, under its ISFM initiatives. Farmers (n=60) were selected randomly from a total of 150 farmers under the SOFECSA cowpea production initiative with the help of local Agricultural Extension Workers (AEWs). Under the SOFECSA program, one main variety of cowpea, CBC2, which is a high-yielding, semi-bushy, short season (60-90 days to maturity) cultivar, has been promoted to eliminate genotypic variation. The farmers planted and managed the cowpea using agronomic recommendations appropriate within their agro-ecological zones (AGRITEX, 1985), with technical support from AEWs and SOFECSA researchers. Appropriate agronomic recommendations included plant spacing of 0.45 m x 0.075 m and application of agro-chemicals to control aphid manifestation during the hot and dry periods of the cropping season. Research approval for this study was obtained from the Department of Agricultural Technical and Extension Services (AGRITEX) of The Government of Zimbabwe's Ministry of Agriculture, Mechanization and Irrigation Development.

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

The amount of mineral fertilizer and organic nutrient resources used by farmers on cowpea were quantified by direct measurements in farmers' fields. This was supported by data collected through a pre-tested questionnaire by interviewing the host farmers. In some cases the amounts were given in local units and then converted to kg ha^{-1}. For example, a standard bucket and scotch cart of cattle manure or woodland leaf litter measured ~20 and 350 kg, respectively. Cowpea grain yield was quantified at physiological maturity from three replicate plots within a field, with each plot measuring 9 m^2. The cowpea fields measured between 0.05-0.4 ha. Harvested cowpea pods were air-dried, shelled and grain yield determined at 9.5% moisture content. A subsample of ~100 g was ground through a 0.5 mm sieve in a stainless steel Thomas-Wiley Model 4 Laboratory mill (Thomas Scientific, Swedesboro, USA) for elemental analysis of Zn.

2.1.2 Selection of fields for experimentation

Soil samples were collected from each cowpea field and analyzed for Zn to guide selection of field experimental sites. A composite soil sample was collected from 10 random points in each field at a depth of 0-20 cm using a Dutch auger. Soil samples were air-dried, sieved through a 2 mm stainless steel sieve and ground to <40 µm in an agate Retsch PM400 Planetary Ball Mill (Haan, Germany). The samples (0.25 g) were digested as described in Joy et al. (2015b) for a broad suite of trace and major elements including total P and Zn in a mixed acid solution (HF 2.5 mL:HNO_3 2 mL:$HClO_4$ 1 mL:H_2O_2 2.5 mL). Subsequent total elemental analyses of the acid digests was carried out by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS; Agilent 7500cx, Santa Clara, USA) in collision cell gas mode (He gas) as described in Hamilton et al. (2015) and Joy et al. (2015b).

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

Based on the results of the preliminary soil analyses, two experimental field sites of contrasting soil physical (texture) and chemical properties were selected in Dendenyore Ward: a sandy soil (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 a low (sandy soil, 0.98 mg kg^{-1}) to adequate (red clay soil, 1.70 mg kg^{-1}) (Table 1) plant available soil Zn status and represented different categories of soil type where cowpea is usually grown on smallholder farms in Zimbabwe, with the sandy soils representing a greater proportion of the surveyed fields. The underlying rationale was that soil texture could potentially influence fertilizer uptake. Both field sites were under an unfertilized cowpea crop during the preceding cropping seasons. It is a common practice by smallholder farmers in Zimbabwe, and elsewhere in southern Africa, to grow grain legumes without any fertilizer input (Kanonge et al., 2015; Snapp et al., 2002).

2.2 Field experiments

2.2.1 Determination of experimental treatments

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

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

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

Guided by earlier SOFECSA work (Kanonge et al., 2015; Manzeke et al., 2014), maize received 10 t organic material ha^{-1} in the 1st year while cowpea received 5 t ha^{-1}. Of the commonly available organic nutrients on-farm (i.e. compost, woodland leaf litter and cattle manure), we only tested cattle manure on cowpea in the 1st season as it is mostly used by farmers (also see Kanonge et al., 2015), but on maize we had treatments with woodland leaf litter and cattle manure because they are the dominant organic nutrients used in maize production (Manzeke et al., 2012). Despite use of sole organic nutrients in cowpea production by some of the surveyed farmers, we deliberately did not include this option because of low P levels in most of the organic nutrient resources, especially cattle manure (Murwira et al., 1995). The low mineral N (16 kg ha^{-1}) and P (14 kg ha^{-1}) treatment, co-applied with locally available cattle manure, was included to cater for farmers who often fail to supply optimal mineral fertilizer to their legume crops. Zinc and organic nutrient resources were only applied in the 1st year of cropping because their residual fertility benefits last up to three cropping seasons (Cakmak, 2008; Mtambanengwe and Mapfumo, 2005).

2.2.2 Establishment and management of the experiment

Land was prepared by conventional ploughing, using an animal-drawn mould-board plough, to a fine tilth before application of fertilizers and planting. Compound D (7N:14 P_2O_5:7K_2O), elemental Zn (applied as $ZnSO_4.7H_2O$ with 22% Zn) and organic nutrient resources were broadcast at planting and then incorporated into the soil by hand hoe. The cattle manure contained 24% organic C, 0.9% N and 29.6 mg Zn kg^{-1} dry weight. Woodland leaf litter had a relatively higher organic C, N and Zn concentrations of 37%, 1.2% and 79.8 mg kg^{-1}, respectively. Thus, application of 10 t ha^{-1} dry weight of the two organic nutrient resources supplied approximately 296 g Zn ha^{-1} (cattle manure) and 798 g Zn ha^{-1} (woodland leaf litter). The total amount of Zn added from organic nutrient resources and mineral Zn fertilizer over the two year cropping period is shown in Table 2. Mineral N and P were supplied to the cowpea crop solely as a basal fertilizer (Compound D) except when applied in combination with organic nutrient resources (see Table 2). For the maize crop, planted at a population density of ~37,000 plants ha^{-1}, ammonium nitrate (AN; 34.5%N) was applied as top dressing in three splits of 30%, 40% and 30% at 2 weeks after emergence (WAE), 6 WAE and at silking, respectively. Cowpea (CBC2) was planted at a spacing of 0.45 m x 0.075 m in triplicate plots measuring 22.5 m^2 to achieve a population of ~296,000 plants ha^{-1}. Weeding was done manually using hand-hoes at 3 and 6 weeks after crop emergence (WAE), resulting in effective control of weeds throughout the growing season. Rogor (Dimethoate 50 EC, Agricura, Harare, Zimbabwe) was used to control aphids in cowpea at a rate of 300 mL ha^{-1}.

2.2.3 Plant shoot biomass and grain yield quantification

Above ground cowpea shoot biomass was quantified at flowering during both cropping seasons using 0.25 m^2 quadrats, from three random sampling points per plot on the sandy soil site. No biomass was collected from the red clay experimental site during the 1st cropping season due to poor germination. The biomass yield was determined on a dry matter basis after oven-drying at 60°C to constant weight. Cowpea and maize grain yields were quantified at physiological maturity from a net plot measuring 10.8 m^2, at a moisture content of 9.5 and 12.5%, respectively. Dried grain samples were ground in a Thomas-Wiley Model 4 Laboratory mill (Thomas Scientific, Swedesboro, USA) to pass through a 0.5 mm sieve. All crop (maize/cowpea) residues were left on the field surfaces, and consumed by livestock during the dry season.

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

The finely ground cowpea grain samples from 1st season experimental plots and selected sample duplications from the farmers' fields were ashed, digested with aqua regia (1 HNO_3: 3HCl) solution and analyzed for total Zn and P using an AAS. Plant Certified Reference Materials (CRMs) used were NIST 1573a (Tomato leaf; National Institute of Standards and Technology, Virginia, USA) and NIST 1567b (Wheat flour; National Institute of Standards and Technology). Colorimetric measurements for P on VIS spectrophotometer and Zn by AAS of plant CRMs provided performance characteristics of 96.7 ±1.9% and 99.6 ±3.1%, for P and Zn, respectively for NIST 1573a and 95.2 ±2.3% and 94.7 ±2.6% for P and Zn, respectively for NIST 1567b.

Grain samples from the 2nd cropping season, CRMs (NIST 1570a, spinach leaves and NIST 1573a tomato leaves; National Institute of Standards and Technology) and blanks were analysed for multi-elements including total Zn and P using a mixed acid (HNO_3 10mL:H_2O_2 1mL) solution in a closed vessel microwave heating system (MARS Xpress, CEM Corporation, Matthews, United States) as described by Joy et al. (2015b). Each analysis of 20 samples included two reagent blank samples, random sample duplications and CRMs for quality control. Subsequent total elemental analysis was carried out by ICP-MS. Performance characteristics for NIST 1570a 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 and Zn, respectively, for NIST 1573a were obtained. To validate elemental Zn and P analysis results obtained using AAS, selected cowpea grain samples from 1st season experimental plots and farmers' fields were re-analyzed using the ICP-MS, and the results were comparable. Nutrient uptake (g ha^{-1}) was quantified on a dry weight basis as the product of nutrient concentration in the grain (mg kg^{-1}) and grain yield (t ha^{-1}).

To estimate Zn bioavailability in humans, the PA to Zn molar ratio was estimated using a 65% grain P conversion ratio (O'Dell et al., 1972; Wu et al., 2009). The subsequent estimated PA:Zn molar ratio was calculated by dividing PA by grain Zn concentration. Zinc absorption is often inhibited by high phytate in grains, a major storage of P which is not digested by monogastric animals including humans (Azeke et al., 2011; Lönnerdal, 2000). A PA:Zn molar ratio >15-20 is considered to hinder efficient absorption of Zn in the digestive tract (Gibson, 2007; Morris and Ellis, 1989).

2.4 Survey and experimental data analyses

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

3. RESULTS

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

The crop survey revealed that more than half of the farmers did not apply any form of mineral fertilizers or organic nutrients to their cowpea crop (Table 3). One third of the farmers applied basal mineral N and P fertilizer at planting, with fertilizer rates ranging from 3.5-30 kg N ha^{-1} (mean = 14) and 0.3-26 kg elemental P ha^{-1} (mean = 8) (Table 3). The fertilizer amounts/quantities applied to cowpea varied by farmer resource endowment. Only 11% of the farmers applied organic nutrient resources in the form of cattle manure, woodland leaf litter or composts, either alone (3%) or in combination with mineral fertilizers (8%). These differences in fertilizer and ISFM strategies by smallholder farmers resulted in differences in grain yield ($P<0.05$) and grain Zn concentration and uptake ($P<0.01$) (Table 4). The largest mean cowpea grain yield of 895 kg ha^{-1} (range = 400-1000 kg ha^{-1}) was obtained when mineral fertilizers and organic nutrients were used in combination. Yields were less when organic nutrient resources (mean=683 kg ha^{-1}) or mineral NPK treatments (mean=566 kg ha^{-1}) were used alone (Table 4). Unfertilized crops gave mean grain yields of less than 300 kg ha^{-1} (range = 40-600 kg ha^{-1}).

The highest cowpea grain Zn concentration of 30.1 mg kg^{-1} was observed when organic nutrients were used in combination with mineral fertilizer, and this corresponded to a grain Zn uptake of 26.9 g ha^{-1} (Table 4). When organic nutrients were used alone, grain Zn concentration was 27.7 mg kg^{-1} and grain Zn uptake was 18.9 g Zn ha^{-1}. The mineral fertilized and the unfertilized crops had the lowest grain Zn concentrations of 24.4 and 23.9 mg kg^{-1}, respectively, and Zn uptakes of 13.8 and 6.8 g Zn ha^{-1}, respectively.

3.2 Contribution of Zn and ISFM to cowpea grain yields

Yields obtained from the field experiments were consistently higher than those under smallholder cropping in the survey (<0.6 t ha^{-1}; Table 4). During the 1st season, cowpea grain yields averaged 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) and sandy soil (Figure 2b), respectively. Zinc fertilizer application did not significantly influence grain yields on the red clay soil ($P>0.05$; Figure 2a). However, on the sandy soil, application of Zn significantly ($P<0.01$) increased grain yields by ~0.2 t ha^{-1} (18%) (Figure 2b). The combination of organic cattle manure, Zn and high rates of mineral N and P increased cowpea grain yields by 38% on the red clay soil and more than doubled to 2 t ha^{-1} on the sandy soil. On the red clay soil, co-application of cattle manure, Zn and a low rate of mineral N and P resulted in cowpea grain yields of 1.7 t ha^{-1} (Figure 2a). Despite a lack of significant differences in cowpea grain yields on the red clay soil, these results were, however, greater than yields of treatments receiving mineral N, P and Zn without cattle manure, which yielded 1.3 t ha^{-1} (Figure 2a).

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

In the 2nd year of cropping, when cowpea followed maize, grain yields ranged between 1.6-1.9 t ha^{-1} and 1.1-1.4 t ha^{-1} on the red clay (Figure 2c) and sandy soils (Figure 2d), respectively. Treatments receiving mineral N and P alone without Zn consistently gave the lowest yields of 1.6 and 1.1 t ha^{-1} on red clay and sandy soils, respectively. There was no effect of Zn application on grain yields on the clay soil ($P>0.05$; Figure 2c). On the sandy soil, application of Zn significantly ($P<0.001$) increased grain yields by 18% compared to plots receiving sole mineral N and P (Figure 2d). On the same site, application of Zn increased grain yields by 16% in plots receiving both mineral and organic (woodland leaf litter) inputs compared to plots receiving mineral and organic woodland leaf litter without Zn (Figure 2d). When Zn was applied to mineral

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

3.3 Effect of Zn on cowpea establishment and shoot biomass yield

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

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

3.4.1 Effect of fertilization on cowpea grain Zn and uptake

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

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

30.2 mg kg^{-1} on sandy soil) when cattle manure and Zn fertilizers were combined with lower 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 combined with cattle manure and Zn applications, grain Zn was 37.4 mg kg^{-1} and 26.2 mg kg^{-1} on the red clay and sandy soils, respectively.

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

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

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

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

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

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

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

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

4. DISCUSSION

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

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

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

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

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

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

While optimal yield benefits were obtained with inorganic Zn fertilizers, application of high quantities of organic nutrient resources in combination with mineral N and P fertilizer, without Zn, can still increase cowpea grain yields (see Table 7; Kanonge et al., 2015). The use of cattle manure and woodland leaf litter has previously been found to give grain yield benefits due to their capacity to supply both Zn (Manzeke et al., 2012) and other nutrients (Giller, 2001) which are essential for legume productivity. This was also evident under field experiments on the sand soil type where cowpea grain yields increased up to 2 t ha^{-1} when 5 t cattle manure ha^{-1} was applied in combination with Zn during the 1st season compared to yields of ~1 t ha^{-1} when Zn was applied without cattle manure (see Figure 2b). Significant increases in cowpea grain yields with cattle manure use could be attributed to organic N supply which enhanced Zn availability, uptake and translocation in the plant as reported earlier in wheat (Kutman et al., 2010; 2011). However, it is unlikely that most smallholder farmers could afford to apply such high levels of organic nutrient resources to grain legumes (Mtambanengwe and Mapfumo, 2009). This lack of capacity to apply large quantities of organic nutrient resources to grain legumes calls for the inclusion of Zn-based fertilizers, and possibly other micronutrients, in the ISFM packages currently being promoted on smallholder farms. There is however, a need to balance N application rates to grain legumes. For example, apparently lower cowpea grain yields attained on the sandy soil during the 2nd season with residual organic N (10 t ha^{-1}) and mineral N application compared to application of 5 t cattle manure ha^{-1} could be attributed to nitrate intolerance in selected grain legumes which could have depressed BNF and crop productivity (Fujita et al., 1992).

4.3 Zinc fertilizer importance in cowpea grain nutrition

Zinc fertilizer application increased grain Zn concentrations of cowpea grown on contrasting soils and treatment combinations, showing its potential to contribute to both crop and human nutrition across variable soils. When cowpea followed the maize crop during the 2nd year of cropping, higher grain Zn concentrations were reported than concentrations attained with direct cowpea fertilization providing evidence of the beneficial effects of legume-cereal rotational systems and possibly increased soil Zn availability and plant uptake within subsequent years of cropping (Manzeke et al., 2014; Wang et al., 2012) . Cowpea is likely to have benefited from the residual fertility from the rotational maize crop which was grown with higher quantities of mineral fertilizers and organic nutrients, as currently practised by smallholder farmers. Maize grain Zn concentrations of up to 35 mg kg^{-1} were attained with Zn fertilization (data not shown). Maize grain Zn concentrations of up to 39 mg kg^{-1} were previously obtained in Zimbabwe following use of Zn-based fertilizers and organic nutrient resources (Manzeke et al., 2014), from a baseline of ~15 to 21 mg Zn kg^{-1} found under sole mineral N and P fertilizer treatments. A higher grain Zn concentration has been reported earlier in cowpea and other grain legumes (Pandey et al., 2013; Poblaciones and Rengel, 2016) compared with maize (Manzeke et al., 2014) and wheat (Gomez-Coronado et al., 2016) grown under similar conditions. These findings show that grain legumes are likely to accumulate more Zn than cereals due to their higher protein content and association between Zn and proteins particularly in the embryo and aleurone of grains (Cakmak, 2000; Kutman et al., 2010). Potentially, more efficient remobilization of Zn from leaves to grains in legumes compared to cereals (White and Broadley, 2009) could also explain the higher grain Zn concentrations in legumes. Other agronomic biofortification methods to increase grain Zn concentration in legumes include pre-soaking of grain legumes and application of foliar sprays (Abdel-Ghaffar, 1988; Cakmak and Kutman, 2017; Ram et al., 2016; Weldu et al., 2012). Using combined approaches and a higher Zn fertilizer rate than one used in this study, it may be possible to meet a target of between 49 and 61 mg kg^{-1}, which are the current targets in field beans and peas, respectively (Bouis and Welch, 2010; Huett et al., 1997). There is therefore a need for constant soils tests to avoid Zn accumulation and probable toxicity effects both in the soil and plants which may be associated with application of higher Zn fertilizers.

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

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

4.4 Potential benefits of Zn fertilizer to dietary Zn intake

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

4.5 Soil type is important when considering agronomic biofortification interventions

Application of Zn fertilizers to cowpea resulted in added grain yield and grain Zn benefits on both soil types, despite marginal increases in yield and grain Zn concentration on the red clay soil. Sandy soils were proportionally more responsive to Zn fertilizers and organic nutrients than red clay soil where insignificant treatment differences in grain yield and grain Zn concentration were reported. Differences in cowpea response to Zn fertilization are likely to be due to differences in soil chemical properties of the two soil types. For example, Zn adsorption increases under high clay content and high pH (Alloway, 2008; Hippler et al., 2015). Tagwira (1991) reported a decrease in $MgCl_2$ extractable Zn with an increase in clay content in similar Zimbabwean soils. The lower specific metal adsorption capacity on sandy soils results in increased plant-availability, and therefore Zn fertilizer use efficiency, than on clay soils. Greater Zn fertilizer response has been reported in citrus trees grown on a sandy loam soil compared to trees grown on a clay soil (Hippler et al., 2015). In addition, Solheim and Solheim (2010) also reported higher maize crop responses on a site with ≤0.5 mg kg^{-1} plant available Zn compared to a site with >1.3 mg Zn kg^{-1}. Based on our findings, Zn fertilizer use efficiency is depended on soil type and geochemistry, which needs to be considered in agronomic biofortification programs. The potential influence of spatial variation in soil type on maize grain micronutrient concentrations and dietary supply has also been reported in Malawian soils (Chilimba et al., 2012; Hurst et al., 2013; Joy et al., 2015b) and other African countries (Sanginga and Woomer, 2009). An improved understanding of soil geochemistry on spatial distribution of micronutrients is therefore important for appropriate and efficient nutrient management on regions and farms which vary in nutrient input requirement for sustainable agriculture and public health interventions.

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

Our findings show benefits of Zn in legume cropping and how beneficial it is for smallholder farmers to use Zn-based fertilizers, and possibly other nutrients, in the dominant legume-cereal cropping systems to enhance food and nutrition security in the face of stress factors such as poor soil fertility and climate change. With recently reported increased changes in rainfall distribution under rain-fed agriculture (Rurinda et al., 2013), enrichment of cowpea with Zn fertilizer and other drought tolerant grain legume crops, often grown in rotation with staple cereals, becomes imperative. Apart from its capacity to fix N in the natural environment, cowpea closely accompanies maize in smallholder cropping and responds well to fertilization. Benefits of Zn fertilizer use on crop productivity and nutrition were apparent in the legume-cereal rotational system, particularly in the legume phase. This concurs with our earlier findings (Kanonge et al., 2015). Higher grain Zn concentration of maize following cowpea (data not shown) in the 2nd season compared to grain Zn concentration attained with direct fertilization of maize implies legume-cereal rotations are a two-way system which complements each other regardless of initially fertilizing the legume or maize. Our current findings show a dimension of enhancing nutritional value of the maize/legume systems which could be employed in soil geochemistry applications.

5. CONCLUSIONS

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

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Tables

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

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 ($mgkg^{-1}$)	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)

[a]LOI was measured as a proxy for soil organic carbon; [b]Mineralizable N after two weeks of anaerobic incubation. Values in parentheses denote standard deviation (SD).

Table 2: Treatments used to determine the influence of mineral and organic fertilizer application and rates on cowpea productivity and grain 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)

[§] implies residual organic cattle manure, woodland leaf litter and Zn fertility in treatments with the respective fertilizers in the preceding maize crop. [†]Denotes 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 K_2O from basal compound D fertilizer. Figures in parentheses denotes amount of elemental Zn (g ha^{-1}) supplied by either 5 or 10 t organic nutrient resource ha^{-1}.

Table 3: Description of fertilization options and fertilizer rates employed in cowpea production by selected farmers during the crop 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 ($7N:14P_2O_5:7K_2O$)
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.

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

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

Treatment	Grain yield kg ha^{-1}	Range kg ha^{-1}	Grain Zn concentration mg kg^{-1}	Range mg kg^{-1}	Grain Zn uptake g ha^{-1}	Range 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	*		**		**	

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

Table 5: Cowpea grain nutritional value under different treatments on a sandy and red clay soil during the 1st cropping season in 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

† 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 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. Figures in parentheses denote standard deviation.

Table 6: Grain Zn content measured in grain collected from experimental sites during the 2nd cropping season in 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	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

* 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 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 did not differ significantly at P<0.05. n/a – not applicable. Figures in parentheses denote standard deviation (SD).

Table 7: A comparison of influence of zinc (Zn) fertilization with other ISFM treatments without Zn on cowpea productivity on sandy 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		

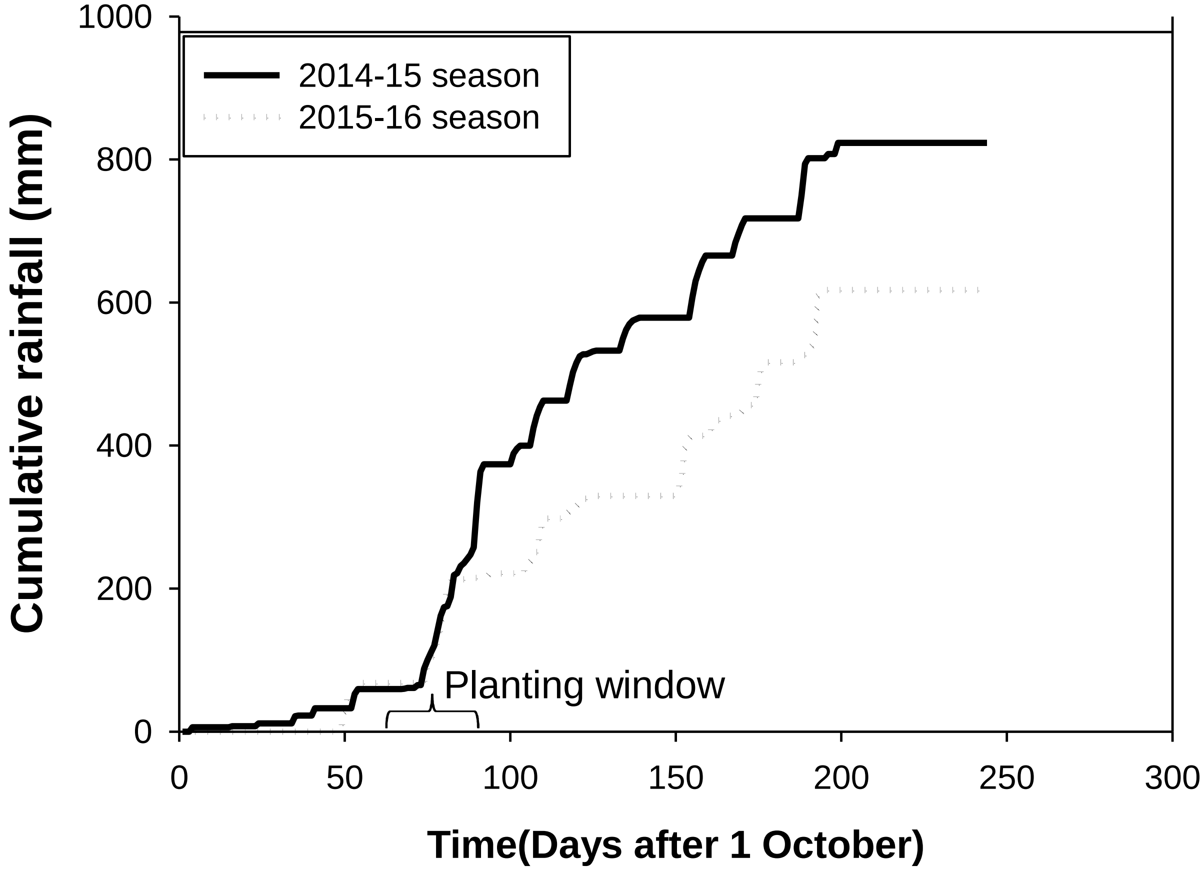
n/a implies not applicable; n/s implies not sampled. †= 1st season sandy soil site treatment used to calculate Zn fertilization benefits on cowpea yield.* = mean excluding the Zn treatment.

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 preceeding 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 preceeding maize crop.



1000
800
600
400
200
0
Cumulative rainfall (mm)
2014-15 season
2015-16 season
Planting window
0
50
100
150
200
250
300
Time(Days after 1 October)

Fig. 1.

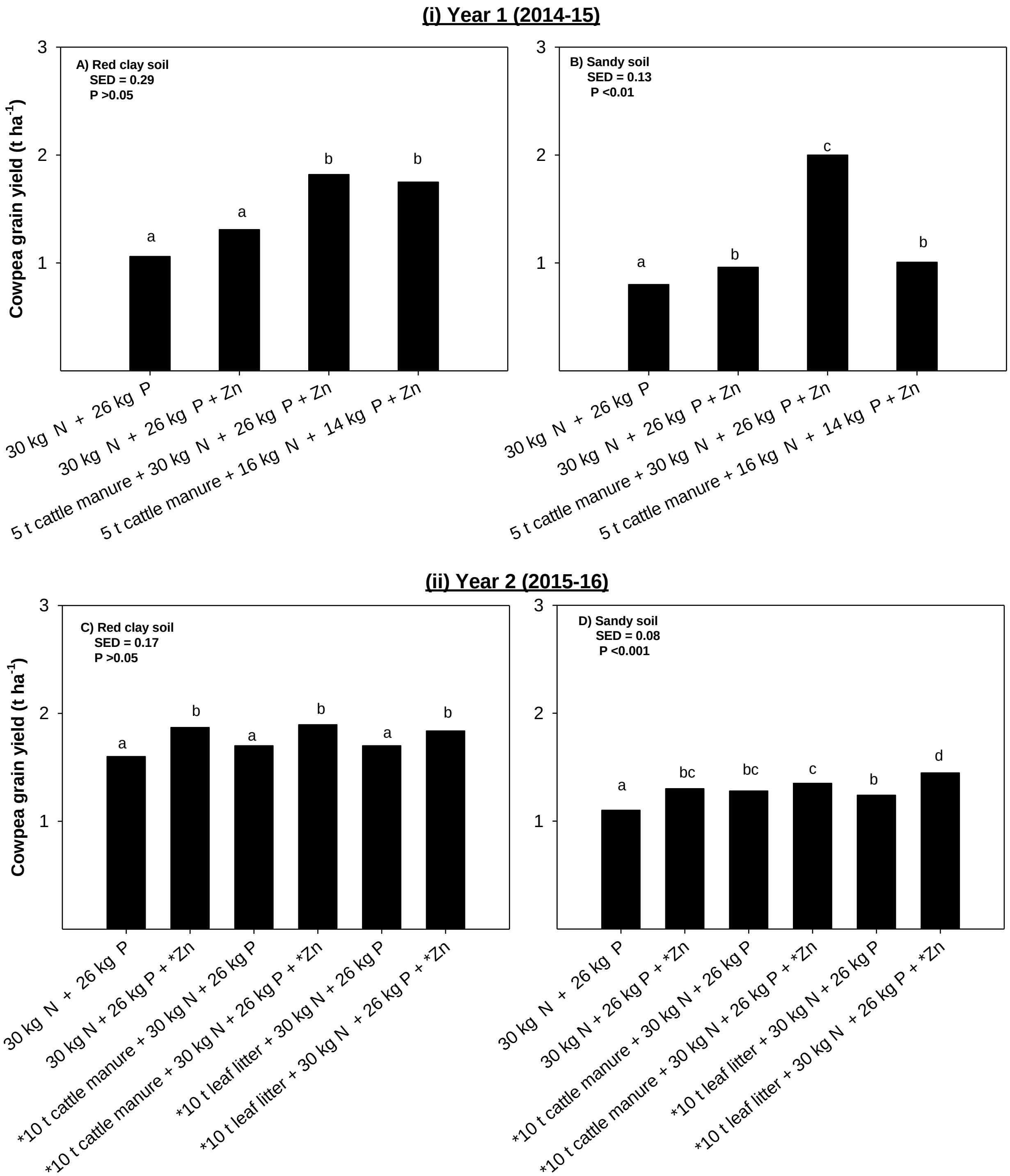

(i) Year 1 (2014-15)
A) Red clay soil
SED = 0.29
P >0.05
B) Sandy soil
SED = 0.13
P <0.01
Cowpea grain yield (t ha-1)
30 kg N + 26 kg P
30 kg N + 26 kg P + Zn
5 t cattle manure + 30 kg N + 26 kg P + Zn
5 t cattle manure + 16 kg N + 14 kg P + Zn
(ii) Year 2 (2015-16)
C) Red clay soil
SED = 0.17
P >0.05
D) Sandy soil
SED = 0.08
P <0.001
Cowpea grain yield (t ha-1)
30 kg N + 26 kg P
30 kg N + 26 kg P + *Zn
*10 t cattle manure + 30 kg N + 26 kg P
*10 t cattle manure + 30 kg N + 26 kg P + *Zn
*10 t leaf litter + 30 kg N + 26 kg P
*10 t leaf litter + 30 kg N + 26 kg P + *Zn

Fig. 2.

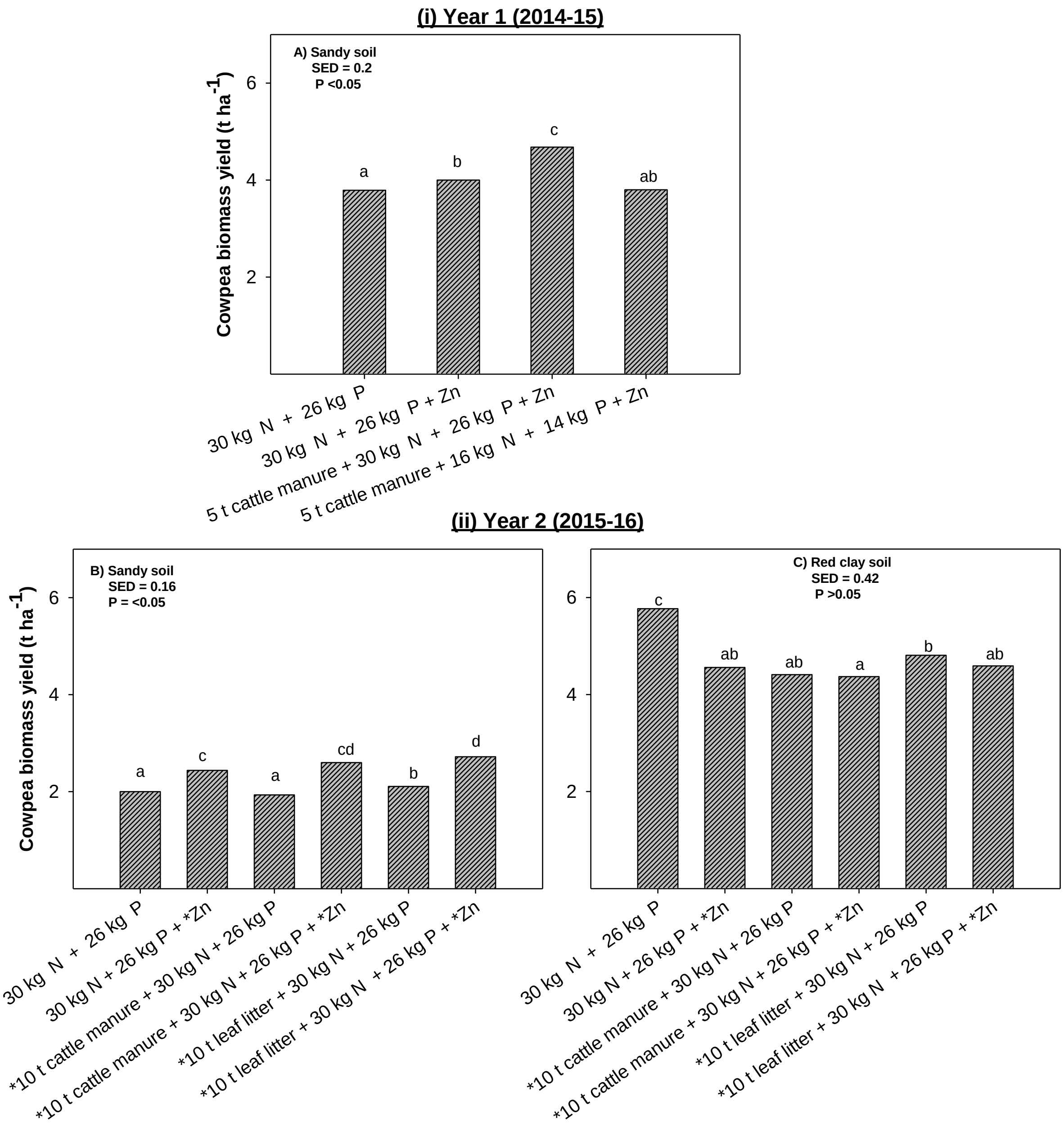

(i) Year 1 (2014-15)
A) Sandy soil
SED = 0.2
P <0.05
Cowpea biomass yield (t ha-1)
6
4
2
a
b
c
ab
30 kg N + 26 kg P
30 kg N + 26 kg P + Zn
5 t cattle manure + 30 kg N + 26 kg P + Zn
5 t cattle manure + 16 kg N + 14 kg P + Zn
(ii) Year 2 (2015-16)
B) Sandy soil
SED = 0.16
P = <0.05
Cowpea biomass yield (t ha-1)
6
4
2
a
c
a
cd
b
d
30 kg N + 26 kg P
30 kg N + 26 kg P + *Zn
*10 t cattle manure + 30 kg N + 26 kg P
*10 t cattle manure + 30 kg N + 26 kg P + *Zn
*10 t leaf litter + 30 kg N + 26 kg P
*10 t leaf litter + 30 kg N + 26 kg P + *Zn
C) Red clay soil
SED = 0.42
P >0.05
6
4
2
c
ab
ab
a
b
ab
30 kg N + 26 kg P
30 kg N + 26 kg P + *Zn
*10 t cattle manure + 30 kg N + 26 kg P
*10 t cattle manure + 30 kg N + 26 kg P + *Zn
*10 t leaf litter + 30 kg N + 26 kg P
*10 t leaf litter + 30 kg N + 26 kg P + *Zn

Fig. 3.