Arbuscular mycorrhizae fungi community structures in Conservation Agriculture soils amended with organic and inorganic fertilizers in a sub-humid region of Zimbabwe

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Abstract

This study investigated AMF community response to tillage and soil amendment regimes from rhizospheric soil of maize roots at 0-10 cm and 10-20 cm at Hunyani farm, Zimbabwe. Two tillage systems (conservation agriculture: CA) and conventional tillage: CT), and six soil fertility amendments [Control (C), High fertilizer (HF-120), Low fertilizer (LF-60), Manure (M), Manure + low fertilizer (MLF-60), Manure + 60 kg N ha^{-1} (M-60)] were laid in a split plot design with three replications.

Twelve morpho-species were identified at the study site. Species richness was highest for CA+C (11.7) and CA+ M (11.7) at 10-20 cm depth. Control plots of CT had more diverse AMF species in the surface layers (Shannon-Weaver index = 2.12) compared to the subsurface soil layer (Shannon-Weaver index = 1.86). The tillage x fertility interaction showed that in CA, *A. dilatata* spore populations were higher in MLF-60 amended plots than all other plots, followed by LF-60 plots, while in CT systems MLF-60 promoted higher spore populations than all other treatments. All plots amended with manure plus inorganic fertilizer had lower ($P<0.05$) *G. clavisprum* spore populations than C, HF-120 and M amended plots under both CA and CT, however LF-20 promoted higher populations than in plots with a combination of manure and inorganic fertilizer in CT only. Tillage x fertility amendment x depth interactions ($P<0.05$) were observed on *A. denticulate, A. schenkii, and C. Luteum, E. infrequens, R. clarus, and S. calospora* spore populations. Medium term effects of CA+ manure, and CA + no amendments may include increasing species richness and diversity. Application of relatively large amounts of inorganic fertilizers increase populations of *C. luteum* populations in CA and decrease in CT. Manure reduces populations of some species e.g. *C. luteum* in both CA and. CT.

Key words: AMF, conventional tillage; fungal hyphae, species diversity; soil fertility amendments, soil health

1. Introduction

Arbuscular mycorrhizae fungi (AMF) have important ecological functions in the soil, and are an important biological indicator of soil quality. Arbuscular mycorrhizae fungi improve crop productivity by stimulating plant uptake of nutrients that include P (e.g. Deguchi *et al*., 2012), and P, Zn, Cu, and Fe in deficient soils (Liu *et al*., 2000; Tarraf *et al*., 2017). Mycorrhizae also improve water uptake (e.g. Zhao *et al*., 2015; Bowles *et al*., 2016), improve aggregate stability (Yang *et al*., 2017) and prevent disease infestation (Azcón-Aguilar *et al*., 2002). Management practises that increase soil disturbance alter soil chemical and physical properties, as well as those that cause changes in vegetation community structure have an effect on the occurrence and community structure of AMF.

Farming systems in most parts of the world, including sub-Saharan Africa (SSA), include conventional tillage (CT) practises and conservation agriculture (CA). Conventional tillage generally involves repeated soil inversion tillage, removal or burial of crop residue, as well as mono-cropping, while CA is a farming system that involves that the simultaneous use of minimum mechanical soil disturbance, maintenance of a permanent or semi-permanent soil cover through mulching, and crop rotation. Conventional tillage in SSA smallholder agriculture is associated with adverse effects on soil health and low crop yields. Meanwhile, CA in most instances improves crop yields (e.g. Rockström *et al*., 2009; Thierfelder *et al*., 2015; Nyagumbo *et al*., 2017). Conservation agriculture (CA) is widely promoted SSA countries (Andersson and D'Souza., 2014; Sithole *et al*., 2016) in order to restore depleted soils and crop yield. CT and CA systems practiced in different SSA soils together with the organic and inorganic soil amendments influence soil AMF community structures. The majority of soils in the smallholder areas of SSA are low in available P due to high soil

67 acidity, high alkalinity and nutrient mining (Nyamangara *et al*., 2000) and are of poor soil
68 fertility.
69
70 Management systems such as tillage, has negative effects on AMF spore populations and
71 alter the community structure (Jansa et al., 2002). Long-term CT has for example been
72 associated with the disappearance of certain AMF species such as *Gigaspora margarita due*
73 to cultivation (Hamel et al., 1994). Meanwhile, inorganic fertilizers are associated with low
74 AMF spore populations (Gosling *et al*., 2006). The population structure, however, vary by
75 region, e.g. the numbers of species (Wang *et al*., 2014; Sale *et al*., 2015; Pontes *et al*., 2017).
76 Medium to long term effects of CA which can contribute to AMF community structure shifts
77 include changes in soil properties, such as soil organic carbon content (Bai *et al*., 2009), soil
78 pH (An *et al*., 2008; Dumbrell *et al*., 2010), P and available N, and soil moisture holding
79 capacity.

80 Arbuscular mycorrhizae fungi are important in soil management and sustainable production.
81 Information on AMF population dynamics in soils across African agricultural systems and
82 effects on different species is however limited. The objectives of this study were: (1) to
83 compare AMF diversity and community composition under two CA and conventional tillage
84 practises (commonly used in sub-Saharan Africa), and (2) to determine effects of soil fertility
85 amendments in a sub humid environment under conventional and CA systems. It was
86 hypothesized tillage system and soil fertility amendments have no effect on the vertical
87 distribution and diversity of AMF in the plough layer.

88 2. Materials and Methods

89 2.1 Site Description

90 This study was conducted at Hunyani Farm, a research station for Chinhoyi University of
91 Technology, (17º21′S, 30^{0}20′E) in Zvimba district, north-western Zimbabwe. The elevation

of site is approximately 1.158 m above sea level. The farm lies in Natural region (NR) IIb of Zimbabwe, with an average annual rainfall of 800-1000 mm and average temperature ranges from 18 to 30 °C. Soils are Chromic Luvisol (WRB, 2014) with a pH (1M $CaCl_2$) of 6.0, and high silt and fine sand content making it prone to surface capping (Kodzwa *et al.*, 2020).

2.2 Experimental Design and field layout

This study was carried as a component of a project on "Developing nitrogen fertilizer recommendations under conservation agriculture in high potential areas". The experiment was established in the 2014-2015 cropping season, and the present study was conducted during the 2018-2019 cropping season, exactly four years after commencement of the experiment. A split plot design was used, with tillage as main factor and mulching (no-mulch and mulch) as the subplot factor, and rotation (rotation and no-rotation) as the sub-subplot factor. Soil fertility treatments as sub-factors. Tillage system, the main factor consisted of Conservation agriculture (CA; reduced tillage+ mulch + rotation of maize and soybean) and Conventional agriculture (CT; plough+ maize mono-cropping + no mulch). Specific soil fertility amendment treatments consisted of six treatments described in Table 1. The manure + low fertilizer treatment (MLF) is a low fertility regime which a middle resourced risk averse farmer would use.

INSERT TABLE 1 HERE

During the sampling season, maize was the crop under production in both rotation and no-rotation plots. Soybean crop grown during the second and fourth season in the sequential rotation system was inoculated with rhizobia, and no top dressing was applied. Maize was planted in basins (2 plants per station) at a target population of 44,444 plants per ha (90 × 50 cm). Herbicides (Basagran) and insecticides (Thiodan) were applied to the crops to control pests and weeds during the experiment.

2.3.1 Soil sampling

Soil samples were collected at maize flowering stage from a 0-10 cm and 10-20 cm depth and a 5 cm×15 cm soil auger was used. Three random sub-samples were collected per plot, combined and thoroughly mixed to form a composite sample. Then sub-samples were then placed in labeled 20 cm x 22 cm Ziploc bags. The samples were stored in a refrigerator at the temperature range of 4 ^{o}C for further processing.

2.4.1 Arbuscular mycorrhizae fungi spore isolation and enumeration

Coarse materials like straw and rocks were removed from the soil samples using a 2 mm-sieve. A 1-g, air-dried soil sample from each composite sample was collected and placed into a glass container with 300 ml of tape water. Spores of AMF were isolated by using the wet sieving and decanting method described by Gerdemann and Nicolson (1963). The soil solution was vigorously mixed using a glass rod for 30 seconds and allowed to settle for 10-15 minutes. The remaining soil-water suspension was slowly poured through sieves of size 40 um, 50 um, 200 um. The sieves were back -washed to extract the spores into a centrifuge tubes. The samples were placed in a centrifuge at 2500 rpm for 5 minutes. 60% sucrose was added to the tubes with the remaining solution and placed in a centrifuge at 1200 rpm for 2 minutes (Daniel and Skipper, 1982) and the suspension was poured into a 10 cm diameter petri dish. The suspension was placed on the 200 um sieve. Water was sprayed and spores collected in a petri dish. Species were observed under a microscope and separated using morphological properties. The number of spores in each group was counted and recorded.

3.4.2. Arbuscular mycorrhizae fungi species identification and enumeration

Clean spores from each group were stained and viewed under a compound microscope with a mounted camera for identification. A drop or two of mountant (polyvinyl lacto glycerol) was spread on the left hand side of a clean and dry slide and Melzer on the right hand side of the slide. Spores were placed on the mountant and the cover slip was placed gently by avoiding

142 air bubbles using a toothpick. Prepared slides were labeled, allowed to dry in a dust free area.
143 Spores were examined under a microscope and photographs taken. Spores were identified
144 based on morphological characterization of spores that is size, color, cell wall layer,
145 ornamentals and hyphae from attached to the spores of the cell wall (Brundet *et al*., 1996;
146 Nusantra, 2012, INVAM, 2015). The INVAM color chat was also used (INVAM, 2015).

147 **2.4 Data Analysis**

148 Arbuscular mycorrhizae fungi diversity parameters (evenness, richness and Shannon-Weaver
149 index) were estimated using Paleontological Statistical package (PAST) version 314
150 (Hammer, 2001). Shannon diversity index (H) was used to evaluate mycorrhizal species
151 diversity, and the diversity index determined using the following formular:

152 $$H = -1 \sum Pi \, In \, Pi,$$

153 Where, H= Diversity Index, Pi = is the proportion of each species in the sample, InPi is the
154 natural logarithm of this proportion (Hutchison, 1970).

155 The value of Shannon and Weaver Diversity Index usually falls between 1.5 and 3.5, only
156 rarely it surpasses 4.5. A value near 4.6 would indicate that the number of individuals is
157 evenly distributed between all species.

158 Arbuscular mycorrhizae fungi population data was Log(x+1.5) transformed to achieve
159 normality of data distribution and homogeneity, but diversity data required no transformation.
160 Both AMF population/abundance and diversity data were subjected to Analysis of Variance
161 (ANOVA) using GenStat Release Discovery Edition (VSN International, 2019) to test for
162 significant differences among the treatments. Where significant differences were detected,
163 mean separation was done using ±standard error of difference (±SED) at the 5% level of
164 significance.

165

3.0 Results

3.1. Arbiscular mycorrhizae community characterisation

Twelve AMF morpho-species were identified at the study site, namely *Scutelospora calospora*, *Rhizophagus clarus*, *Acaulospora denticulate*, *Acaulospora dilatata*, *Entrophospora infrequens*, *Rhizophagus intraradices*, *Claroideoglomus luteum*, *Funneliformis mossae*, *Paraglomus occultum*, *Archaeospora schenckii*, *Glomus clavisporum*, and an unidentified species. Across all tillage system × soil fertility amendment combinations, *F. Mossae* was the most abundant species with relative abundances of 15-25%, followed by *R. intraradices* (5-10%). *S. calospora* was absent in most CT treatments except in M, and MLF-60 treatments, while the species was absent in one CA treatment i.e. CA+ C (control; Figure 1).

3.1 Arbuscular mycorrhizae fungi spore populations

Both tillage system and soil fertility amendment had significant effects ($P < 0.001$) on AMF spore densities (Table 2). However, these main effects were confounded by significant tillage system × soil fertility amendment interaction ($P < 0.05$) on AMF spore counts at flowering stage in maize (Figure 2).

INSERT TABLE 2 HERE

The significant interaction of tillage system and soil fertility amendment revealed that in CA+ MLF-60 had the highest total AMF population, followed by M-60, M and control, while LF-60 and HF-120 had significantly lower spore populations than other CA treatments. Meanwhile, in CT, MLF-60 and control had higher total AMF spore populations than all other soil fertility amendments.

3.2 Arbuscular mycorrhizae fungi species diversity

3.2.2 Treatment effects on AMF species richness, evenness, and diversity within the soil layer

Tillage had a significant effect ($P < 0.05$) on species richness and on diversity index (Shannon – Weaver index; Table 2). Soil amendment type had a significant effect ($P<0.001$) on species richness and on diversity index but not on species evenness. However, these effects were confounded by tillage x soil amendment x depth effects. There were significant effects ($P < 0.05$) of tillage × fertility × depth interaction on AMF species richness and Shannon-Weaver Index; (Figure 3a-b). In CT, control plots (no soil fertility amendments) contained significantly ($P < 0.05$) higher AMF species richness in the top 0-10 cm than the underlying 10-20 cm soil layer (Figure 3a). For the Shannon-Weaver index, results showed that control plots of CT had more diverse AMF spore populations in the surface layers (Shannon-Weaver index = 2.12; Figure 3b) compared to the subsurface soil layer (Shannon-Weaver index = 1.86). In contrast, AMF spore populations in control plots of CA were less diverse (Shannon-Weaver index = 2.20) than the underlying 10-20 cm soil layer (Shannon-Weaver index = 2.35).

There was a significant ($P < 0.001$) effect of depth on species evenness (Table 2). There was significant ($P < 0.05$) of tillage × fertility interact on AMF species evenness (Figure 3c). In CA, the application of HF resulted in a significant increase ($P < 0.05$) in AMF species evenness from 0.85 (LF) to 0.92. Meanwhile there were no depth effects on AMF diversity for all other treatments. There was significant effect ($P < 0.05$) of tillage × fertility interaction on AMF species evenness (Figure 3c).

3.3 Arbuscular mycorrhizae fungi species abundance

3.3.1 Tillage, soil amendments and depth effects on species abundance

There were significant tillage effects ($P< 0.05$) on all species, except *A. spinosa and G. clavisporum* (Table 3). However, for all the pecies except *R. intraradices,* the effects were

218 confounded by interactions of factors. *R. intraradicles* spore populations were higher (P <
219 0.01) in CA plots than in CT plots (Table 4).
220
221 **INSERT TABLE 3 HERE**
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223 **INSERT TABLE 4 HERE**
224
225 There were significant (P< 0.001) soil fertility effects on all species (Table 3). However, for
226 all species with the exception of R. *intraradices*, the effects were confounded by interactions.
227 *R. intraradicles* spore populations were highest in MLF-60 plots and lowest in C (control)
228 untreated plots (Table 4).
229
230 There were depth effects (P< 0.05) on all species except for *S. schenkii* (Table 2). However,
231 the effects were confounded by interactions for all species except for R. *intraradices* which
232 had more spore populations in surface soil layers relative to 10-20 cm soil depth (Table 4).

233 **3.3.2 Tillage × depth interactions on species abundances**
234 Tillage x depth effects were significant (P < 0.05) for S. schenkii, *C. luteum, F. mossae* and
235 *P. occultum*. However, depth x tillage effects on *S. schenkii* and *C. luteum* were confounded
236 by tillage x depth x soil amendment interactions (Table 3). In both CA and CT systems,
237 *F.mossae* and *P. occultum* spore populations were higher in top 10 cm but the magnitude of
238 the difference was higher in CA for *F. mossae,* and higher in CT for *P. occultum* (Figure 4a-
239 b).

240 **3.3.3 Soil fertility amendment × depth interactions on species spore populations**
241 Soil amendment x depth effects were significant (P < 0.05) for AMF community structures
242 under Conservation agriculture with organic and inorganic soil amendments in sub-humid
243 region of Zimbabwe. *A. dilatata*, *C.luteum*, *F.mossae*, *G. clavisporum, R. clarus and S.*
244 *calospora,* the significant effects on *A. denticulate and C. luteum, R. clarus* and *S. calospora* were

however confounded by tillage x depth x soil amendment interactions (Table 3). *A. dilatata* spore populations were higher in top 10 cm compared to 10-20 cm of LF-60 and M-60 amended plots, but there were no differences for the other treatments (Figure 5a). *F. mossae* spore populations were higher in the top 10 cm across all soil fertility treatments but largest difference was in HF-120 amended plots (Figure 5b). HF-120 and M amendments resulted in higher *G. clavissporum* spore populations in top 10 cm compared to 10-20 cm, and all other soil fertility amendment had no effect on the vertical distribution of this species (Figure 5c).

3.3.4 Tillage × fertility interaction on different AMF species

There was a significant tillage × fertility interaction ($P < 0.01$) on A. denticulate, A. dilatata, *A. spinosa, A. shenckii, C.lutem, E. infrequens, F. mossae, G. clavisporum, R. clarus, and S. calospora* (Figure 6). Interactions were confounded by tillage x depth x soil amendment interactions for *A. denticulate, A. shenckii, C. lutem, E. infrequens, R. clarus, and S. calospora* (Table 3). In both CA and CT plots, *A. dilatata* spore populations were higher in MLF-60 amended plots than all other plots and lowest in HF-120 amended plots (Figure 6a). However, in CA plots, LF-60 plots had higher populations than C, M, and M-60 plots but populations in these treatments were similar in CT plots. *A. spinosa* spore populations were higher in MLF-120 plots and significantly lower in C and HF-120 plots compared to all other CA plots (Figure 6b). Meanwhile in CT plots, MLF-120 had higher populations and M-60 plots had lower populations than all other treatments. *F. mossae* spore populations were significantly higher in MLF-120 plots, followed by LF-60 plots, then M-60 under both CA and CT (Figure 6c). However, under CT, HF-120 and C had similar populations but in CA systems, HF-120 had higher spore populations than C plots. The manure + fertilizer combinations i.e. MLF-60 and M-60 combinations resulted in lower spore populations than other plots for both CA and CT, in addition to LF-60 for CA plots. *G. clavisporum* spore populations were significantly lower in manure-amended plots i.e. M, MLF-60, and M-60,

plots under both CA and CT tillage systems (Figure 6d). In addition, LF-60 plots had similar populations to these treatments in CT plots. Populations were significantly higher in HF-120 plots in CT while LF-60 had higher spore populations than other treatments in CA plots. Higher in LF-60 plots, and In CT plots, the populations of the species were highest in HF-120plotsfollowed by control plots.

3.3.5 Tillage × fertility × depth interactions

Tillage × fertility amendment x depth interactions ($P < ,0.05$) were observed on *A. denticulate*, *A. schenkii*, *C. luteum*, *E. infrequens*, *R. clarus* and *S. calospora* spore populations (Table 3). In CA plots, C, LF-60, M and M-60 amendments resulted in higher spore populations of *A. denticulate* in the 10-20 cm compared to 0-10 cm soil depth (Figure 7a). Meanwhile, in CT, C, LF-60, and M-60 plots promoted higher spore populations of *A. denticulate* in the 10-20 cm than the top 10 cm soil depth, while M did not. Higher *A. schenkii* spore populations were observed in the surface layers of CT + MLF-60 relative to the underlying 10-20 cm soil depth, while there no depth effects in soils with other amendments (Figure 7b). Meanwhile, in CA plots, MLF-60 had higher spore populations at 10-20 cm compared to 0-10 cm. CA + HF-120, CT + control and CT + HF-120 had the lowest *C. luteum* spore populations. *C. Luteum* populations were higher in surface layers compared to subsurface layers in CT+HF-120 under both CA and CT, as well as in LF-60 amended plots in CA systems (Figure 7c). All plots which had manure had low C. luteum populations in both CA and Ct systems. *E. infrequens* populations were higher in sub-surface layers compared to surface layers in the following treatments for CA; HF-120, MLF-60 and M-60, and in CT for plots amended with LF-60, M, MLF-60 and in plots with no amendments (Figure 7d). There were no depth effects for the other treatments. Soil fertility amendments did not result in a depth effect on *R. clarus* populations in CA plots (Figure 7e). Meanwhile CT plots amended with M had lower surface populations while surface

populations were higher than subsurface populations in plots amended with MLF-60 , and in plots with no amendments (C) (Figure 7e). *S. calospora* populations were higher in surface soil compared to subsurface soil (10-20 cm) in CA+HF-120, LF-60, M, and CT plots with MLF-60. HF-120 and LF-60, C and M-60 had negative effects on *S. calospora* populations (Figure 7f).

4.0 Discussion

Treatment effects on AMF spore abundance

The tillage x fertility amendment type interaction resulted in higher AMF spore counts in CA+ MLF-60 plots than any other treatments. CA promotes higher spore populations than CT in many agro-ecosystems (Sale *et al*. 2015 and de Pontes *et al*., 2017). Soil disturbance in conventional tillage destroys fungal hyphae thereby affecting growth and development (Galvet *et al*., 2001; Jansa *et al*., 2002) and thus contribute to low spore populations in CT systems compared to CA. Furthermore, mulching, practised in CA, improves root colonization by fungi by preventing soil mycelium disruption and increasing AMF propagule abundance (Verzeaux *et al*., 2017). Thus, higher spore counts in CA + manure treatments compared to CT + manure treatments can be explained by the combined effects of reduced tillage, mulching, and rotation with a mycotrophic crop i.e. soybean, which promotes AMF fungi.

There were lower total AMF spore populations in soils treated with relatively high fertilizer rates compared to plots amended with manure and those with low fertilizer rates. Thus inorganic fertilizer had negative effects on spore populations. This can be explained by negative effects of the relatively high amounts of P and/or high N. High application of N fertiliser especially long term fertilizer application reduces AMF spore populations (Johnson et al., 2003; Miao-Yan et al., 2009). High fertilizer rates increase soil P

concentrations, which inhibit AMF spore formation and reduce external hyphae of AMF in the soil (Gosling et al., 2006) or/and increase soil N which decrease dependence of plants on mycorrhizae. Some species were however, promoted by high fertilizer rates.

Treatment effects on AMF spore diversity

Twelve species of AMF were identified with AMF species richness ranging from 8.3 to 10.4 across plots with different soil fertility amendments. Similarly, 19 AMF species from six genera in semi-arid steppes of China (Wang *et al*., 2014), and 15 AMF morphospecies from eight genera were identified in depleted soils in Benin (Johnson *et al*., 2013). However, higher soils in other agro-ecological regions had either higher numbers (Sale *et al*., 2015; Tchabi *et al*., 2008; Säle, Aguilera *et al.,*), or lower in some soils (Pontes *et al*., 2017).

F. mossae was the most abundant species in most of the treatments at the study site, except for CT + no amendments plots (negative control) and CT + High fertilizer plots) treatments, where *G. clavisporum* was the dominant AMF species. The study site has clay soils, which are known to suitable for development and growth of *F. mossae* (Sari *et al*., 2004). Meanwhile, *S. calospora* was mainly associated with CA systems in this study, and absent in most conventional tillage plots.

CA promoted high species richness particularly in treatments with manure and with no amendments than in CT systems. Pontes *et al*. (2017) similarly found lower species richness in conventionally tilled systems (12–17 species), when compared to no-tillage (15–18 species). In CT plots, treatments with HF-120 and M-60 had the lowest AMF species richness. Lower species richness in plots with high fertilizer rates for both CA and CT indicate negative effects of fertilizers on AMF growth in these soils. Of note is that *A. spinosa*, *A. dilatata*, and *C. luteum* AMF spore populations were negatively affected by inorganic fertilizers in soils at the study sites. de Pontes *et al*. (2017) showed that not P or

344 organic C, but pH and available cations, affected AMF species richness, hence the effects of
345 high fertilizers on species richness in this study. The high species richness in top soil
346 compared to lower depth in CT+C (no amendment) may be due to more soil disturbance in
347 the soil and soil chemical properties.

348 The higher species diversity observed in top soil than in subsoil (10-20 cm) in conventional
349 plots in our soils could be due to a more stressed soil environment. There was higher AMF
350 species diversity in CA + control at 10-20 cm, CA + low fertilizer (10-20 cm) and CA +
351 manure (10-20 cm) than all CT plots. Lower species diversity index in CA plots with high
352 fertilizer compared to other CA plots could be associated with higher available phosphorus
353 levels. Johnson *et al*., (2013) showed that high available phosphorus has negative effects on
354 AMF species diversity and evenness while soil organic carbon did not have an effect on AMF
355 diversity indices.

356 Species evenness was similar between CA and CT systems, except in plots with LF-60,
357 where AMF species evenness in CA was higher than that of CT plots. High species evenness
358 in CA+ LF-60 plots than all other plots could be indicating a more established environment.
359 Pereira *et al*., (2018) found that diversity, evenness and richness indices were higher in an
360 environment under greater stress (in their case with crop rotation) while diversity, species
361 evenness and richness indices tended to be lower in communities established in climax
362 environments (Pereira *et al*., (2018). They proposed that this increased mycorrhizal symbiosis
363 could be a strategy by which fungi and plants overcome biotic and abiotic stresses that occur
364 in the soil.

365 **Main treatment effects on species abundance**

366 CA promoted high *R. intraradices* spore populations than CT system. Generally spore
367 populations decrease with depth (Muleta *et al*., 2008; Oehl *et al*., 2005; Yang *et al*., 2010),

368 and this was noted for *R. intraradices* whose spore populations decreased with depth
369 irrespective of tillage system or type of soil fertility amendment. Meanwhile, MLF-60
370 amendments resulted in high *R. intraradices* spore populations while C (no amendments
371 resulted in the least spore populations.

372 **Tillage x depth interaction on species spore populations**

373 Generally, spore populations decrease with depth. *F, mossae and P. occultum* populations
374 were higher in top 10 cm of soil for both CA and CT, the magnitude of difference being
375 higher in CA than CT for *F. mossae*. CA mulching promotes spore formation thus could have
376 a contribution to the observed difference.

377 **Tillage x fertility amendment effects on species spore populations**

378 Sole application of inorganic fertilizer (HF-120), as well as sole application of manure
379 promoted high *G. clavisporum* AMF spore populations while combining manure and
380 inorganic fertilizer resulted in low populations of *G. clavisporum* in both CA and CT system.
381 High populations in CT+C and CT+HF-120, suggest that *G. clavisporum* can survive in
382 conditions promoted by nutrient depletion that may include low pH. The HF-120 and control
383 systems (no amendment) promoted the highest populations in Ct, while in CA manure had
384 the largest impact on populations. In contrast, *A dilatata* populations were depressed by high
385 fertilizer and promoted the most by a combination of manure and low fertilizer (MLF-60)
386 under both CA and CT tillage, and followed by LF-60 in CA plots. Similarly, MLF-60 also
387 promoted more spore populations of *A. spinosa* than the control. However, in this case soils
388 amended with inorganic fertilizer had higher populations than control plots in CT plots. *F.*
389 *mossae* populations were promoted by all fertilizer amendment types in CA, while in CT HL-
390 120and control plots had similar populations. *F. mossae* is a generalist AMF species that can
391 survive under different conditions in terms of fertility and type of tillage system, are adapted

392 to varied fertility amendments and they survive in both acidic and alkaline soils (Pando and
393 Tarafdar, 2004). The ability of *F. mossae* to survive and proliferate in different environments
394 was demonstrated by results from this study. Effects of tillage x fertility interactions on *F.*
395 *mossae* and *A. spinosa* population dynamics appear to be similar.

396 **Fertilizer x depth effect on species spore populations**

397 While spore populations generally decrease with depth, *A. dilata*, *F. mossae*, and *G.*
398 *clavisporym* populations were not affected by depth for some soil fertility amendments in the
399 top 20 cm in soils. There was no depth effect on *G. clavisporium* spore populations in soils
400 amended with LF-60, MLF-60 and M, while only HF-120 and M-60 resulted higher A. dilate
401 populations at 0-10 cm compared to 10-20 cm. Furthermore, higher rates of manure or
402 fertilizer appear to promote high populations of *G. clavisporum*. Meanwhile, *F. mossae*
403 populations decreased with depth under all amendment types though the magnitude of the
404 difference was highest in soils amended with high fertilizer rates.

405 **Tillage x soil amendment x depth effect on species populations**

406 The three way interactions show that species vary in their response to depth, under various
407 tillage and soil amendment types. *A. denticulate* populations, populations increased with
408 depth for most treatments except in CA+ HF-120, and CA+MLF-60 and in CT plots amended
409 with HF-120, M or MLF-60. These treatments in which there was no depth effect also had the
410 lowest populations suggesting negative effects of these treatments i.e. high fertilizer rates,
411 manure+low fertilizer, in CA and Ct systems as well as Manure in CT plots on A. denticulate
412 populations. Meanwhile, *A. schenkii* populations either increased or decreased with depth or
413 were not affected by depth depending on tillage and soil amendment type. MLF-60 and M-60
414 in CA plots resulted decrease in populations with depth while the opposite was true CT plots

amended with MLF-60. Similar to findings for A. denticulate, HF-120 had negative effects on populations and the treatments had no depth effect in both CA and CT systems.

C. luteum populations' response to inorganic fertilizers for the different tillage systems were opposite to those of *A. schenkii* and *A. denticulate*. LF-60 promoted high populations of C. luteum in CA plots while high fertilizer promoted high populations in CT plots, but for both treatments, populations decreased with depth. Manure amendments whether applied sorely or in combinations with inorganic fertilizers appear to depress populations of *C. luteum*.

S. calosprora populations greatly vary by tillage system, the populations were low in CT plots with no amendments, or with inorganic fertilizer (HF-120, LF-60 and M-60) and were not affected by depth, while the populations were promoted by MLF-60 and decreased with depth in Ct systems. Meanwhile, in CA systems both organic and inorganic fertilizers and their combinations had positive effects on populations, decreasing with depth in HF-120, LF-60, and M amended plots. Therefore, inorganic fertilizers have negative effects on *S. calospora* populations in CT but positive effects on CA tillage systems.

E. infrequens generally showed a pattern of increase of populations with depth, with significant effects being observed in the following Ca plots, HF-120, MLF-60, and M-60, while for CT populations increased with depth except for plots amended withHF-120, and M-60. Meanwhile, there were no depth effects on *R. clarus* spore populations for all fertility treatments under CA, while spore populations in CT plots were lower at lower depths compared to surface soils in C, MLF-120, and M amended plots. Soil disturbances in surface layers of CT may have positive effects on spore populations for this species. The inversion of soil under high tillage may distribute surface spores to lower depths (Yang et al., 2010).

Vertical variation in community composition has been noted in some studies (Muleta et al., 2008; Oehl et al., 2005; Yang et al., 2010). Oehl et al. (2005) found that many AMF species

439 would only sporulate in deeper horizons, below tilled depths. The species populations are
440 negatively influenced by high fertilizer while low fertilizer, and manure amendments have
441 positive effects.

442 The species' different behavior under various tillage, fertility amendments, and depth show
443 variability in ability to grow and survive in different environmental stressors. Knowledge of
444 each species requirement is important in developing management strategies for sustainable
445 soil health.

446 5.0 Conclusion

447 Tillage systems, soil fertility amendments, and depth have effects on species diversity and
448 spore populations in Hunyani soils of Zimbabwe. The soils have at least 12 species of AMF,
449 with the most dominant species being *F. mossae,* while *S. calospora* is mainly associated
450 with CA systems in under these soils and agro-climatic conditions. High fertilizer rates lower
451 species richness while manure combined with CA generally promotes species richness. CA +
452 Manure application +low fertilizer rates, similar to those that can be applied by low –
453 resourced smallholder farmers in sub-Saharan Africa, promote species diversity as well as
454 CA with no amendment (being higher at 0-10 cm compared to 10-20 cm). *R intradicles* AMF
455 spore populations increase with depth; their spore populations are promoted by CA, and by
456 manure + low fertilizer rates. Meanwhile, high fertilizer rates depress *A. dilatata* spore
457 populations in both CA and CT systems. In contrast, *G. Clavisporum* spore populations are
458 promoted by high fertilizer rates, and manure in each case applied as sole amendments, while
459 combining the inorganic and organic fertilizers have negative effects on populations
460 compared to control plots. Sole manure application decreases A. denticulate populations, in
461 CT systems, while it promotes higher populations in CA particularly at lower depths. *A.*
462 *schenkii* populations decreased with high fertilizer application. Manure addition either as sole

amendment of in combination with inorganic fertilizer reduces populations of *C. luteum*, while sole inorganic fertilizers promoted populations particularly in the surface layers in CT plots, while LF-60 has the highest impact in CA systems. Inorganic and organic amendments increase *S. calospora* populations in CA systems but inorganic fertilizers (sole) depress in CT systems.

Data accessibility

Upon acceptance of the manuscript, all the data that support the findings of this study will be openly available in Dryad.

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576

577 Table 1: Soil fertility treatments

Treatment	Description
Control (C)	No soil fertility amendment was applied.
High fertilizer (HF-120)	Inorganic fertiliser applied at a rate 120 kg N, 63 kg P_2O_5 and 31.5 kg K_2O ha^{-1}.
Low fertilizer (LF-60)	Inorganic fertiliser applied at a rate 60 kg N, 31.5 kg P_2O_5 and 15.75 kg K_2O ha^{-1}.
Manure applied (M)	Manure was applied at 10 tons/ha.
Manure + low fertilizer (MLF-60)	Manure at 10 tons/ha + inorganic fertiliser applied at a rate 60 kg N, 31.5 kg P_2O_5 and 15.75 kg K_2O ha^{-1}.
Manure + 60 kg N ha^{-1} (M-60)	Manure applied at 10 tons/ha + inorganic fertiliser applied at a rate 60 kg N ha^{-1}.

578

579 Table 2: Effect of treatments on AMF species spore populations and diversity during the
580 2018/19 cropping season.

	Tillage effect	Soil amendment effect	Depth effect	Tillage x soil amendment	Tillage x depth effect	Soil amendment x Depth effect	Tillage x soil amendment x Depth effect
Total AMF spore populations	***	***	NS	*	*NS*	*NS*	*NS*
Species richness	*	***	NS	***	*NS*	*NS*	*
Shannon-Weaver index	*	***	*	***	*NS*	*NS*	***
Species evenness	NS	NS	***	*	*NS*	*NS*	*NS*

581
582
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584 Table 3: Effect of treatments on AMF species spore populations and diversity during the
585 2018/19 cropping season.

	Tillage effect	Soil amendment effect	Depth effect	Tillage x soil amendment	Tillage x depth effect	Soil amendment x Depth effect	Tillage x soil amendment x Depth effect
A. denticulate	**	***	***	***	***NS***	***	**
A. dilatata	*	***	**	*	NS	*	NS
A. spinosa	NS	***	***	***	NS	NS	NS
A. schenckii	***	***	NS	**	*	*NS*	*
C. luteum	*	***	***	***	**	***	***
E. infrequens	**	*	***	*	*NS*	NS	*
F. mossae	**	***	***	**	*	*	*NS*
G. clavisporum	ns	***	***	*	ns	***	*NS*
P. occultum	*	***	***	ns	*	ns	*NS*
R. clarus	*	***	*	***	*NS*	***	*
R. intraradices	**	***	***	*NS*	*NS*	*NS*	*NS*
S. calospora	**	***	***	***	*	*	*

586 * P≤0.05 **P≤0.01 P≤0.001

587 Table 4: Effect of treatment factors on *R. intraradices* at Hunyani farm, Zimbabwe, during
588 the 2018/19 cropping season. Data was transformed using log(x+1.5).
589

Treatment Factor		*R. intraradices*
Tillage	CA	0.8551[b]
	CT	0.6662[a]
	P –value	*<.01*
	±s.e.d	*0.01424*
Fertility amendment	Control	0.5499[a]
	HF-120	0.6743[b]
	LF-60	0.8994[d]
	M	0.7113[bc]
	MLF-60	0.9778[e]
	M-60	0.751[c]
	P –value	*<.001*
	±s.e.d	0.03634
Depth	0-10 cm	0.8786
	10- 20 cm	0.6427
	P –value	*<.001*
	±s.e.d	0.01471

590 For each species, means followed by the same letter within columns are not significantly
591 different based on ±standard error of difference (SED, P ≤ 0.001).