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Influence of Farmyard Manure Application on Potential Zinc Solubilizing Microbial Species Abundance in a Ferralsol of Western Kenya

Peter Bolo [1,2,*], Monicah Wanjiku Mucheru-Muna [2], Romano Kachiuru Mwirichia [3], Michael Kinyua [1], George Ayaga [4] and Job Kihara [1]

[1] Alliance of Bioversity International and International Center for Tropical Agriculture (CIAT) c/o, International Centre of Insect Physiology and Ecology (ICIPE), Duduville Campus Off Kasarani Road, Nairobi P.O. Box 823-00621, Kenya
[2] Department of Environmental Sciences and Education, Kenyatta University, Nairobi P.O. Box 43844-00100, Kenya
[3] Department of Biological Sciences, University of Embu, Embu P.O. Box 6-60100, Kenya
[4] Kenya Agricultural and Livestock Research Organization (KALRO), Nairobi P.O. Box 30148-00100, Kenya
* Correspondence: p.bolo@cgiar.org

Abstract: Zinc is an important nutrient for plant growth and development. Its availability is influenced by zinc solubilizing microbes (ZSMs). The effects of commonly promoted agronomic practices on the abundance of ZSMs are so far not well understood. In this study, conducted in 2019, we assessed the effects of farmyard manure (FYM) application, either sole or in combination with residue and/or inorganic fertilizer inputs, on ZSM community structure using 11 treatments in a long-term (17 years) integrated soil fertility management experiment located in Western Kenya. Bacterial and fungal community composition were evaluated by amplicon sequencing on an Illumina MiSeq platform. The results showed that putative ZSMs (i.e., the ZSMs generally considered to possess the zinc solubilizing capabilities) were clustered in two major clades based on either the application or no application of FYM. Sole application of FYM significantly ($p < 0.05$) increased the abundance of several ZSMs under a maize–Tephrosia rotation. In addition, systems with the combined application of FYM with other inputs generally showed significantly increasing trends for some ZSMs under a maize–Tephrosia rotation. Moreover, the combined application of FYM and P rather than only P significantly increased the abundance of some ZSMs under maize monocropping systems. Furthermore, as well as affecting ZSM abundance, soil chemical variables involving soil organic carbon (SOC), total N and Olsen P significantly increased with FYM application. This study indicated that management practices such as the application of FYM that increase SOC, and other soil chemical parameters, also/concomitantly increase ZSM abundance. These results imply enhanced capacities for microbial-linked zinc availability with FYM application.

Keywords: farmyard manure; zinc solubilizing microbes; microbial diversity; abundance; soil DNA sequencing; agricultural inputs; long-term agronomic management

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1. Introduction

Zinc (Zn) is one of the most important micronutrients in plants. It catalyzes several metabolic enzyme reactions [1] and promotes photosynthesis, plant resistance to environmental stresses, the formation of pollen and proteins, the metabolism of nitrogen and carbohydrates and the production of antioxidants [2]. Among the micronutrients, soil Zn deficiency, reaching about one-third of arable soils, is the most frequent challenge globally [3], affecting crop and animal health and productivity [4]. Zn deficiency has been linked to hidden hunger [5], manifested through malnutrition-associated disorders.

Zinc exists in soil as both water-soluble and insoluble complexes, with the major portion being non-bioavailable for plant uptake [6]. The bioavailability of zinc depends on

soil physicochemical conditions such as the concentration of zinc in solution, its interactions with other nutrients and ion speciation, plant root exudates and microbial activities [7–9]. Increasing soil P availability, as influenced by long-term fertilization, may compromise Zn uptake by plants due to the antagonistic nature of the two nutrients, with potential adverse effects on crop yield and nutritional quality [10]. On the other hand, the application of nitrogen fertilizer can increase plant uptake of P and Zn from soil [11]. Zinc deficiency results in poor plant growth, yields and nutritional quality arising from depressed zinc-associated metabolic processes [6]. Remedying soil zinc deficiency and promoting crop productivity calls for agronomic interventions with the potential for enhancing zinc bioavailability, both at lower costs and in an ecofriendly manner. The conventional application of synthetic Zn-based fertilizers is not only less efficient and non-ecofriendly but also associated with high fertilizer costs [12].

In the recent past, a lot of attention has been directed towards the potential of zinc solubilization by soil microbial communities [12–15] to remedy soil zinc deficiency. The soil microbes that are capable of solubilizing zinc from insoluble sources are generally referred to as zinc solubilizing microbes (ZSMs) [12,14]. In addition to zinc solubilization, ZSMs can enhance the capabilities of plants to uptake Zn from the soil, thus improving the enrichment of Zn in the edible plant parts [12–15]. The ZSMs, existing either in bacteria or fungal nature, may increase the bioavailability of Zn to plants from insoluble sources [16,17] by employing different biological processes involving chelation, chemical transformation and the production of organic acids [1,18,19]. This makes ZSMs potential alternatives for enhancing Zn bioavailability and supplementation for plants in a sustainable, ecofriendly and cost-effective manner [12,14]. Fungal strains belonging to the genera *Penicillium, Glomus, Aspergillus*, *Trichoderma* and *Beauveria* have been identified as prominent fungal ZSMs [12,20,21], whereas the most promising bacterial strains have been identified in the genera *Azospirillum*, *Burkholderia, Serratia*, *Agrobacterium*, *Bacillus*, *Rhizobium*, *Thiobacillus*, *Gluconacetobacter* and *Pseudomonas* among others [1,14,22].

As well as ZSMs, the addition of micronutrient-based fertilizers, breeding of zinc-efficient germplasm, seed biofortification, introduction of beneficial soil microorganisms and correction of soil alkalinity alongside the adoption of crop rotation practices can also alleviate soil zinc deficiency [23–25]. Addressing soil-based Zn deficiencies can reduce food insecurity and hidden hunger that affects at least 30% of the global human population [26,27].

Regenerative agriculture practices involving crop rotation, the application of organic manure and the reduced use of inorganic fertilizers have been widely promoted to improve soil health and fertility and sustain agricultural productivity [28–30]. The application of manure, either alone or in combination with inorganic fertilizers, is an agronomic practice with great potential to improve Zn availability in the soil. Manure use is a traditional soil fertility management practice employed by many smallholder farmers in sub-Saharan Africa, especially those with a mixed crop–livestock system in place [31,32]. If manure is properly managed, manure application can improve soil fertility, crop yields and soil biological properties through the provision of multiple nutrients [33–35].

However, despite the benefits of such agricultural management practices on improving soil fertility and crop yields, little is known on their effects on zinc solubilizing microbial species, especially in tropical soils. In this study, we assessed how regenerative agriculture practices such as manure and crop residue applications, with or without fertilizer applications, affect the abundance of zinc solubilizing microbial species in a tropical agricultural soil.

2. Materials and Methods

2.1. Study Site

This research was carried out in October 2019 (during the short rains period) in a long-term agronomic trial named INM3 in Madeya, Siaya County, Kenya. The trial was established in 2003 by the International Center for Tropical Agriculture (CIAT) and lies between 0.14° N and 34.40° E, under a subhumid climate with a biannual rainfall of 1200–1600 mm and an average temperature of 23.2 ± 1.5 °C [36]. Soils in the trial site are Ferralsols, characterized by low pH (5.1 ± 0.3), with a sand–silt–clay ratio of 15:21:64 and extractable inorganic phosphorus (Olsen) content of 2.99 ± 2.09 mg kg^{-1} [36–38]. Crop production in the region is mainly for subsistence, rain-fed and mostly practiced under conventional tillage in smallholder farms (mostly less than 1 ha), with maize being the dominant staple food crop grown mostly as an intercrop with common beans. Plant residue retention is less common as a large proportion is grazed on after harvest, and only a few farmers incorporate manure in the soil, but in considerably low quantities and at inconsistent intervals.

2.2. Experimental Design

The experiment was initiated in 2003 under a split plot design with 48 treatments replicated four times, out of which 11 were selected for this study. The main plots were two farmyard manure rates (0 and 4 t ha^{-1} FYM application), subplots were three cropping systems (maize and Tephrosia (i.e., *Tephrosia vogelii*) rotation, maize and soybean intercropping and continuous maize), sub–subplots were two residue application rates (with or without 2 t ha^{-1} residue application). There were four rates of N fertilization (0, 30, 60 and 90 kg N ha^{-1}) as urea, two rates of P (0 and 45 kg P ha^{-1}) as triple superphosphate (TSP) and blanket application of K (at 60 kg ha^{-1}) as muriate of potash during planting. The whole experiment has 192 plots each measuring 4.5 m by 6 m.

Within the trial, maize and Tephrosia were planted at a spacing of 25 cm by 75 cm and in a rotation system, with 2 seeds placed per hill and later thinned to one. Soybean was intercropped with maize, at a spacing of 5 cm by 75 cm. Urea was applied in two splits, whereby one-third was applied during planting and two-thirds applied during topdressing. Farmyard manure was incorporated in the field during planting. The chemical properties of the farmyard manure used in the study were as follows; pH 6.48, electrical conductivity (4.04 mS/cm), dry matter (94.8%), carbon (13.6%), N (1.18%), P (0.29%), K (0.488%), Ca (0.803%), Mg (0.323%), S (0.11%), Mn (2319 ppm), Fe (48,050 ppm), Zn (128.5 ppm), Cu (42.975 ppm), B (50.45 ppm), Na (458.25 ppm) and C:N (11.5). Hand ploughing and weeding using hoes were restricted to 15 cm depth.

2.3. Selection of Treatments

Eleven (11) treatments representing different management practices under conventional tillage were selected for the study (Table 1). The 11 treatments were selected to confer the effects of certain input combinations embedded in different management practices (under either continuous maize cropping or maize–Tephrosia rotation) on putative zinc solubilizing microbes.

2.4. Soil Sampling and Analysis

Soil samples were collected 8 weeks after planting at 0–15 cm depth in October 2019 during the short rains period. Maize was the main crop during sampling. Samples were taken from five spots per plot within each treatment following a "W" shaped pattern, using an auger. The samples were transferred in a bucket, thoroughly mixed, representative samples packed in labeled zip-lock bags, kept in a cool box with frozen ice packs and transported to the laboratory for processing and further analyses. The samples for biological assessments (except microbial biomass C) were sieved (2 mm) and stored frozen (−20 °C) until extraction. The samples for various chemical analyses were air-dried before grinding

and sieving. Samples for microbial biomass C assessment were immediately sieved (2 mm) and analysed right after sampling from the field.

Table 1. Description of the treatments selected for the study in INM3 Madeya experimental site.

Code	Treatment Abbreviation	Treatment Description	Cropping System	FYM	Res	N (Kg ha^{-1})	P (Kg ha^{-1})
Inm1	None	No input	MT	-	-	0	0
Inm2	P + N	NP fertilizer	MM	-	-	60	45
Inm3	R only	Residue	MT	-	+	0	0
Inm4	FYM only	FYM	MT	+	-	0	0
Inm5	P + N + R	NP fertilizer + residue only	MM	-	+	60	45
Inm6	FYM + PN + R	NP fertilizer + residue + FYM	MM	+	+	60	45
Inm7	FYM + PN	NP fertilizer + FYM only	MT	+	-	60	45
Inm8	FYM + R	Residue + FYM only	MM	+	+	0	0
Inm9	P only	P fertilizer only	MM	-	-	0	45
Inm10	P + FYM	P + FYM only	MM	+	-	0	45
Inm11	P + N *	90N + P fertilizer only	MM	-	-	90	45

All the treatments were under conventional tillage. MT = maize and Tephrosia rotation; MM = continuous maize; + = with; - = without; Res = residue, applied at 2 Mg ha^{-1}; FYM = farmyard manure applied at 4 t ha^{-1}. All treatments were applied with 60 kg K ha^{-1}; * denotes N applied at 90 kg ha^{-1}.

2.5. Soil Chemical Analyses

Soil organic carbon and total N were determined by the Carbon Nitrogen (CN) Elemental Analyzer. Soil pH was determined in water (soil–water; 1:2), while, except for Olsen P, the exchangeable cations (Ca, Mg, K, Zn, S, Fe, B, Mn, Na and Al) were extracted following the Mehlich (1984) procedure, and concentrations were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

2.6. Determination of Microbial Biomass Carbon

Microbial biomass C was determined using the MicroBiometer Soil Test kit (https://microbiometer.com (accessed latest on 12 January 2021)). Briefly, fresh soil samples were sifted. Using a calibrated syringe, 1 mL of the samples were taken, compacted to 0.5 mL and the excess soil removed from the tip of the syringe. One sachet of the provided powder from the kit was transferred into a clean tube, water added and briefly mixed using a whisker. Soil samples were added, briefly mixed with the contents, whisked for 30 s to fully mix with the fluid and allowed to settle for 20 min. After every five minutes, the contents were briefly tapped to allow the floating debris to settle down the tube. After 20 min settling time, the samples were extracted using a pipette and 3 drops carefully applied to the reading card without wetting the surrounding of the card. The readings for microbial biomass carbon were thereafter taken by imaging the card using the MicroBiometer App (Version 3.6.0) from Google Play Store.

2.7. Deoxyribonucleic Acid (DNA) Extraction from Soil

Total DNA was extracted from 0.2 g of the fresh soil samples using phenol–chloroform–isoamyl (PCI) alcohol as described in [39]. Briefly, 0.2 g of soil was suspended in 200 µL of solution A (containing 100 ul Tris-HCl (pH 8.0), 100 mM EDTA (pH 8.0), vigorously vortexed, 5 µL of Lysozyme (20 mg/mL solution) added and the mixture incubated in a water bath (370 °C) for 30 min. A total of 400 µL of lysis buffer (containing 400 mM Tris-HCl (pH 8.0), 60 mM EDTA (pH 8.0), 150 mM NaCl and 1% sodium dodecyl sulfate) was added and incubated at room temperature for 10 min. Thereafter, 10 µL of Guanidinium thiocyanate (GITC; for protein denaturation) was added and the mix incubated (65 °C)

in a water bath for 2 h. An equal volume of phenol–chloroform–isoamyl was added, mixed briefly at 13,200 rpm for 15 min at 4 °C and the supernatant containing the crude DNA transferred to a new tube. To the supernatant was added 150 µL of sodium acetate and 600 µL isopropyl alcohol (2-propanol), mixed briefly by inversion and left at room temperature for 10 min. This was followed by centrifugation at 13,200 rpm for 10 min to pellet the DNA. The supernatant was discarded and the resultant DNA pellets washed in 300 µL of 70% ethanol two times. The DNA pellets were air-dried and thereafter dissolved in 30 µL of PRC water. DNA quantity and quality was checked on 1% agarose gel electrophoresis against a 1 Kb marker. The DNA pellets were lyophilized and shipped to MRDNA labs (www.mrdnalab.com (accessed on 12 January 2021), Shallowater, TX, USA) for amplicon generation and sequencing.

2.7.1. Soil DNA Sequencing, Bioinformatics Sequence Processing and Taxonomic Identification

The PCR amplification of the 16S rRNA gene V4 variable region was carried out from extracted DNA generated from rRNA, using barcoded bacteria/archaeal primers 515/806 with barcode on the forward end (i.e., 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′)) as previously described by [40]. Briefly, PCR amplicons were generated using HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, and a final elongation at 72 °C for 5 min. Illumina DNA library preparation and sequencing was performed following the manufacturer's guidelines. The raw reads were preprocessed using a proprietary pipeline by the sequencing facility. Briefly, the sequences were joined, followed by the removal of barcodes, sequences with ambiguous base calls and those with fewer than 150 base pairs. This was followed by denoising, the generation of operational taxonomic units (OTUs), the removal of chimeras and the clustering of OTUS at 3% divergence (97% similarity). Finally, the OTUs were taxonomically classified using BLASTn against a curated database derived from RDPII and NCBI (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu).

2.7.2. Identification of the Potential Zinc Solubilizing Microbial Species

Since the study majorly aimed at understanding the general overview of the agronomic management effects on potential zinc solubilizing microbes, we conducted an in-depth literature review to identify and list the potential soil microbial genera known to solubilize zinc (ZSM). The search was conducted in Google Scholar and Web of Science search engines, with the timeline restricted to returning outcomes only up to the year 2022. The resultant files were reviewed and the potential zinc solubilizing microbes reported in the articles listed. Using this list of potential zinc solubilizers, we then identified and selected those microbes from the already available Illumina data. Thus, the potential ZSM genera reported herein are those most commonly documented by several previous papers. We further acknowledge that for some of the potential ZSM genera reported herein, although they have the potential for solubilizing zinc, they may also perform other functions in the soil.

2.8. Statistical Data Analysis

Microbial diversity analysis was conducted using the Vegan package in R Project. Alpha diversity (microbial diversity within samples) was estimated using Shannon and Chao 1 diversity indices to test the significant differences between the different agronomic management systems. Beta diversity (β diversity, diversity between samples) was used to test the significant differences between samples. This was determined by computing the principal component analysis (PCA) of the ZSM versus the treatments using PAST software (v.4.05) [41]. The data were Logarithm$_{10}$ transformed before performing PCA to meet the assumptions of parametric tests [42]. The determination of Bray–Curtis similarities and principal component analysis (PCA) were also carried out using PAST software (v.4.05).

Hierarchical cluster analysis (HCA), analysis of variance (ANOVA), canonical correspondence analysis (CCA) and non-metric dimensional scaling (NMDS) were performed using R project. ANOVA was performed on square root transformed data. Mean separation was performed using Tukey's HSD at $p \leq 0.05$. Canonical correspondence analysis (CCA) was used to assess the relationship between the soil microbial community richness and soil chemical parameters. The CCA analysis was performed using the *Anacor* library and *cca* function in Vegan library in R, overall significance was tested using the *anova* function, and the *step* function was used to determine significant variables with a permutation test at 999 maximum permutations.

3. Results

3.1. Effects of Management Practices on Soil Chemical and Physical Characteristics in INM3 Site

Agronomic management practices significantly affected the soil chemical and physical parameters. Soil pH, SOC, total N, Mg, Zn, Fe and CEC were significantly ($p \leq 0.05$) higher under the sole application of FYM (Inm4) compared to the no input application (Inm1) or the application of inorganic fertilizer only (Inm2; Table 2). The application of inorganic fertilizer only (Inm2) significantly increased ($p \leq 0.05$) soil P (Olsen) and S but significantly reduced ($p \leq 0.05$) soil pH relative to the no input application (Inm1). The highest pH was in the treatment with the combined application of FYM and crop residues followed by all other FYM treatments, while the no input and inorganic fertilizer treatments had the lowest pH.

Table 2. Effects of management systems on soil chemical and physical characteristics in INM3 site in the year 2019.

Treatment	pH	SOC (%)	N (%)	P (mg kg^{-1})	Mg (mg kg^{-1})	Mn (mg kg^{-1})	S (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})	Zn (mg kg^{-1})	Fe (mg kg^{-1})	EC *	CEC #
Inm1	4.84 ^{de}	1.70 ^{cd}	0.13 ^{ef}	18.1 ^{e}	37.5 ^{d}	394 ^{ab}	17.1 ^{d}	5.26 ^{c}	0.13 ^{cd}	1.00 ^{c}	118 ^{c}	31.7 ^{b}	6.54 ^{d}
Inm2	4.51 ^{f}	1.64 ^{d}	0.12 ^{f}	79.0 ^{a}	37.5 ^{d}	431 ^{ab}	22.1 ^{bc}	5.51 ^{bc}	0.17 ^{bcd}	1.30 ^{c}	128 ^{bc}	43.6 ^{ab}	6.84 ^{d}
Inm3	4.88 ^{de}	1.83 ^{bcd}	0.14 ^{def}	13.0 ^{e}	45.3 ^{d}	400 ^{ab}	19.0 ^{cd}	5.62 ^{abc}	0.18 ^{abcd}	1.23 ^{c}	117 ^{c}	32.0 ^{b}	6.64 ^{d}
Inm4	5.16 ^{abc}	2.05 ^{a}	0.17 ^{ab}	23.8 ^{de}	122.3 ^{ab}	396 ^{ab}	19.8 ^{cd}	5.92 ^{abc}	0.23 ^{abc}	2.61 ^{ab}	143 ^{b}	42.0 ^{ab}	11.8 ^{ab}
Inm5	4.66 ^{ef}	1.68 ^{cd}	0.13 ^{f}	71.2 ^{ab}	36.7 ^{d}	441 ^{a}	23.3 ^{ab}	5.65 ^{abc}	0.12 ^{cd}	1.28 ^{c}	133 ^{bc}	44.0 ^{ab}	6.71 ^{d}
Inm6	5.06 ^{bcd}	1.96 ^{ab}	0.16 ^{bcd}	65.6 ^{ab}	138.7 ^{a}	397 ^{ab}	21.1 ^{bc}	6.29 ^{ab}	0.19 ^{abcd}	3.57 ^{a}	169 ^{a}	52.2 ^{a}	12.9 ^{a}
Inm7	4.95 ^{cd}	1.86 ^{abc}	0.15 ^{cde}	55.9 ^{bc}	126.7 ^{ab}	366 ^{b}	23.7 ^{ab}	6.12 ^{abc}	0.17 ^{bcd}	3.29 ^{a}	174 ^{a}	50.4 ^{a}	12.6 ^{a}
Inm8	5.43 ^{a}	2.07 ^{a}	0.18 ^{a}	20.5 ^{e}	152.3 ^{a}	420 ^{ab}	21.8 ^{bc}	6.37 ^{a}	0.29 ^{a}	3.46 ^{a}	126 ^{bc}	42.5 ^{ab}	12.1 ^{ab}
Inm9	4.98 ^{cd}	1.70 ^{cd}	0.13 ^{ef}	56.7 ^{bc}	89.8 ^{bc}	389 ^{ab}	24.0 ^{ab}	5.85 ^{abc}	0.17 ^{bcd}	1.84 ^{bc}	126 ^{bc}	42.7 ^{ab}	10.1 ^{bc}
Inm10	5.32 ^{ab}	1.83 ^{bcd}	0.16 ^{bc}	59.2 ^{abc}	161.5 ^{a}	405 ^{ab}	26.2 ^{a}	6.23 ^{ab}	0.27 ^{ab}	2.58 ^{ab}	139 ^{bc}	47.8 ^{a}	13.0 ^{a}
Inm11	4.54 ^{f}	1.74 ^{cd}	0.14 ^{ef}	42.5 ^{cd}	55.4 ^{cd}	425 ^{ab}	24.3 ^{ab}	5.77 ^{abc}	0.12 ^{d}	1.61 ^{bc}	129 ^{bc}	48.2 ^{a}	8.62 ^{cd}

Values with similar letters in each column are not significantly different. EC = electrical conductivity of salts; CEC = cation exchange capacity; * = uS/cm; # represents meq/100 g; Inm1 = no input; Inm2 = P + N fertilizers only; Inm3 = residue only; Inm4 = +FYM only; Inm5 = P + N + residue only; Inm6 = P + N + FYM + residue only; Inm7 = P + N + FYM only; Inm8 = residue and FYM only; Inm9 = +P fertilizer only; Inm10 = P + FYM only; Inm11 = P + 90 kg N ha^{-1} only. Inm1–Inm8 are all under maize–Tephrosia rotation system. Inm9–Inm11 are all under maize monocropping system.

The simultaneous application of FYM, NP fertilizers and residue (Inm6) significantly increased ($p \leq 0.05$) soil pH, SOC, total N, Mg, Zn, Fe and CEC relative to the combined application of NP fertilizers and residue only (Inm5). The combined application of P and FYM (Inm10) significantly increased ($p \leq 0.05$) soil pH, total N, Mg, B and CEC relative to the sole application of P (Inm9) under the continuous maize system. Integrating both P and 90 kgN ha^{-1} (Inm11) fertilizers in a continuous maize system (Table 2) significantly reduced soil pH compared to the sole application of P (Inm9). This is perhaps due to the increases in the soil acidity effect associated with urea nitrogen application.

The management systems without FYM application had zinc concentrations that ranged between 1.0 and 1.84 mg kg^{-1}. However, all the systems where FYM was added solely or in combination with other nutrients had zinc concentrations that ranged between

2.61 and 3.57 mg kg^{-1}. The highest zinc concentrations (3.29–3.57 mg kg^{-1}) were obtained in systems where FYM was added in combination with full NPK fertilization.

Olsen P negatively correlated with total N, SOC and K but positively correlated with available S, Fe and EC. Available Zn, Cu, B and CEC positively correlated with total N, SOC, pH, available K, Ca and Mg (Table 3). CEC positively significantly correlated with the soil characteristics. Total N positively correlated with SOC, pH, available K, Ca, Mg, Cu, B, Zn and CEC, and a similar correlation (as that of total N) was true for the variables relating to SOC, pH, available K, Ca and Mg. These correlations indicate that the management systems such as the application of FYM that increase SOC, also (concomitantly) increase several soil chemical parameters involving the soil pH, total N, available K, Ca, Mg, Cu, B, Zn and CEC.

Table 3. Correlation between different soil chemical characteristics in INM3 site in the year 2019.

	P †															
P †	1	N (%)														
N (%)	−0.43 *	1	SOC (%)													
SOC (%)	−0.43 *	0.85 **	1	pH												
pH (1:2) ₩	−0.34	0.76 **	0.70 **	1	K †											
K †	−0.40 *	0.76 **	0.72 **	0.80 **	1	Ca †										
Ca †	−0.10	0.71 **	0.54 **	0.82 **	0.58 **	1	Mg †									
Mg †	−0.10	0.70 **	0.73 **	0.85 **	0.70 **	0.87 **	1	Mn †								
Mn †	0.07	0.15	0.07	−0.02	−0.11	−0.04	−0.07	1	S †							
S †	0.45 *	−0.02	−0.18	−0.01	−0.16	0.32	0.25	−0.11	1	Cu †						
Cu †	−0.07	0.44 *	0.54 **	0.49 **	0.46 **	0.56 **	0.65 **	0.01	0.26	1	B †					
B †	−0.30	0.73 **	0.60 **	0.72 **	0.54 **	0.69 **	0.63 **	0.23	0.11	0.43 *	1	Zn †				
Zn †	−0.02	0.73 **	0.70 **	0.65 **	0.62 **	0.67 **	0.84 **	−0.04	0.23	0.54 **	0.42 *	1	Fe †			
Fe †	0.36 *	0.19	0.30	0.14	0.21	0.29	0.41 *	−0.14	0.10	0.28	−0.09	0.56 **	1	Na †		
Na †	0.13	−0.01	0.24	0.21	−0.05	0.38 *	0.41 *	−0.04	0.18	0.38 *	0.08	0.18	0.26	1	EC	
EC ¥	0.43 *	0.18	−0.05	−0.10	−0.02	0.23	0.19	−0.22	0.47 **	0.28	−0.04	0.40 *	0.47 **	0.09	1	CEC
CEC #	0.06	0.67 **	0.60 **	0.67 **	0.45 **	0.85 **	0.82 **	0.08	0.28	0.60 **	0.53 **	0.74 **	0.55 **	0.45 *	0.41 *	1

CEC = cation exchange capacity; EC = electrical conductivity; SOC = soil organic carbon; * represents significant correlation at $p \leq 0.05$ level; ** represents significant correlation at $p \leq 0.01$; † represents mg kg^{-1}, ¥ = uS/cm; # represents meq/100 g, ₩ represents soil–water.

3.2. Overall Microbial Abundance and ZSM Species Abundance in INM3

Overall, 2,113,815 and 2,219,424 high-quality reads for bacteria and fungi, respectively, were obtained. The 2,113,815 high-quality bacterial sequences were clustered into 691 OTUs at 97% genetic distance, which were further assigned to 36 phyla, 87 classes, 161 orders and 298 families. Similarly, the 2,219,424 high-quality fungal sequences were clustered into 721 OTUs at 97% genetic distance, and were further assigned to 27 phyla, 79 classes, 191 orders and 379 families.

The ten most dominant bacterial phylotypes at phylum level were Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria, Gemmatimonadetes, Chloroflexi, Planctomycetes, Verrucomicrobia, Bacteroidetes and Nitrospirae, whereas the most abundant fungal phylotypes (in order from highest to lowest genera) were Mortierella, Fusarium, Penicillium, Gymnochlora, Sclerostagonospora, Rhizophlyctis, Alternaria, Myceliophthora, Cochliobolus, Humicola and Pseudofavolus. Out of 691 bacterial and 721 fungal phylotypes (at genus level), 31 potential zinc solubilizing microbial genera were identified from the Illumina data based on the outcomes ZSM genera obtained from the literature reviews. Fifteen (15) out of the 31 potential ZSM genera identified in the INM3 site (Figure 1) had relative abundance greater than 0.5%. The 15 potential ZSM genera comprised of *Penicillium* spp., *Burkholderia* spp., *Bacillus* spp., *Sphingomonas* spp., *Rhizobium* spp., *Bradyrhizobium* spp., *Trichoderma* spp., *Glomus* spp., *Aspergillus* spp., *Thiobacillus* spp., *Arthrobacter* spp., *Azospirillum* spp., *Lysinibacillus* spp., *Pseudomonas* spp. and *Mesorhizobium* spp. in INM3.

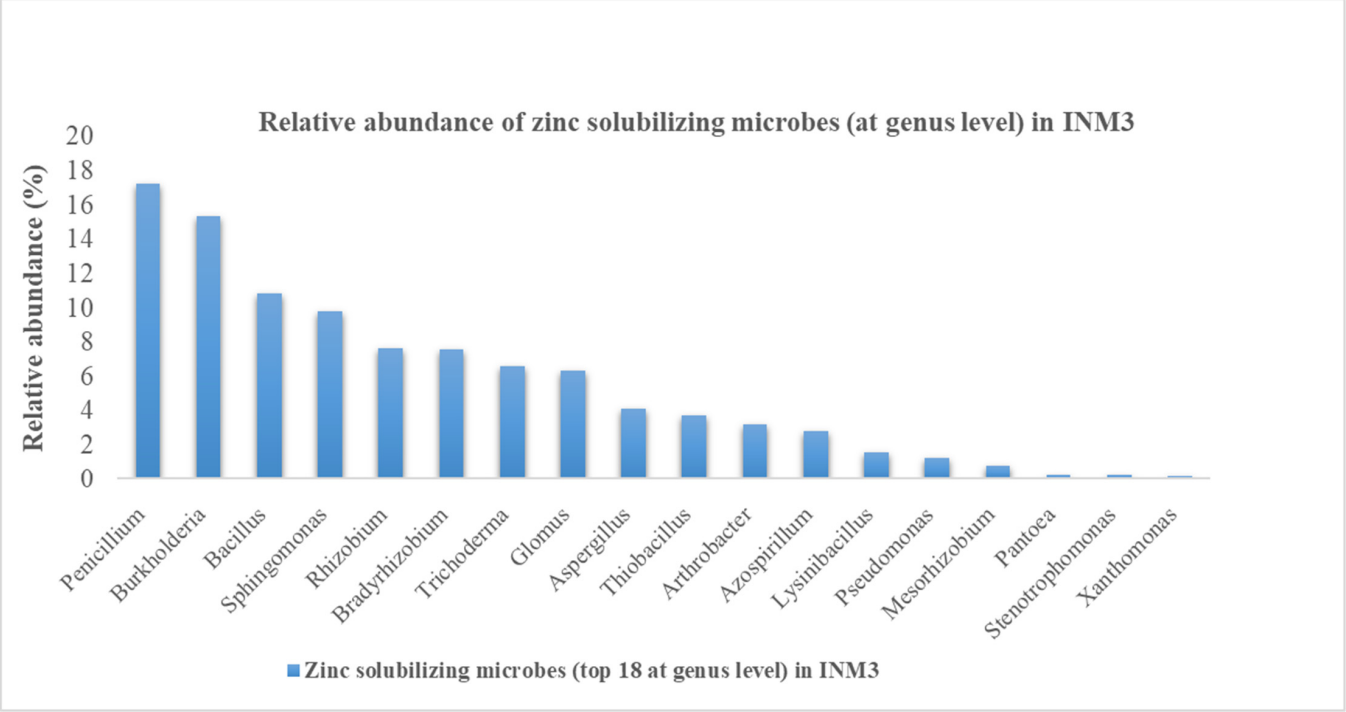

Figure 1. Relative abundance (>0.5%) of zinc solubilizing microbial species in INM3 site in the year 2019.

3.3. Effect of Agronomic Management Practices on ZSM Species

Bray–Curtis similarity cluster analysis revealed distinct grouping of treatments based on the management practices implemented. The results in the dendrogram (Figure 2) showed that the ZSM species were clustered in two major clades based on either the application or no application of farmyard manure (i.e., management practices having FYM addition were grouped in one major clade and those without FYM in a different clade (Figure 2: HCA results)). The subsequent clades emanating from each of the two major clades showed that the species in the different management practices were not quite homogeneous, although either the application of, or lack of, FYM similarly affected them.

The clustering of the management practices with FYM addition had an almost 91.4% similarity index (Bray–Curtis), while those lacking FYM addition had 90.0% (Figure 2). Management practices with the combined application of P, N and residue were similarly grouped (92.5% similarity index), and the same case was observed under the treatments with no input application, and the sole application of either residue or P (all under a 92.3% similarity index).

A shift in the distribution of ZSM was observed in the integrated soil fertility management under the practice with the application of FYM relative to practices lacking FYM addition (Figures 3a,b and 4a,b). However, cropping systems had negligible influence on ZSM species distribution (Figures 3a,b and 4a,b).

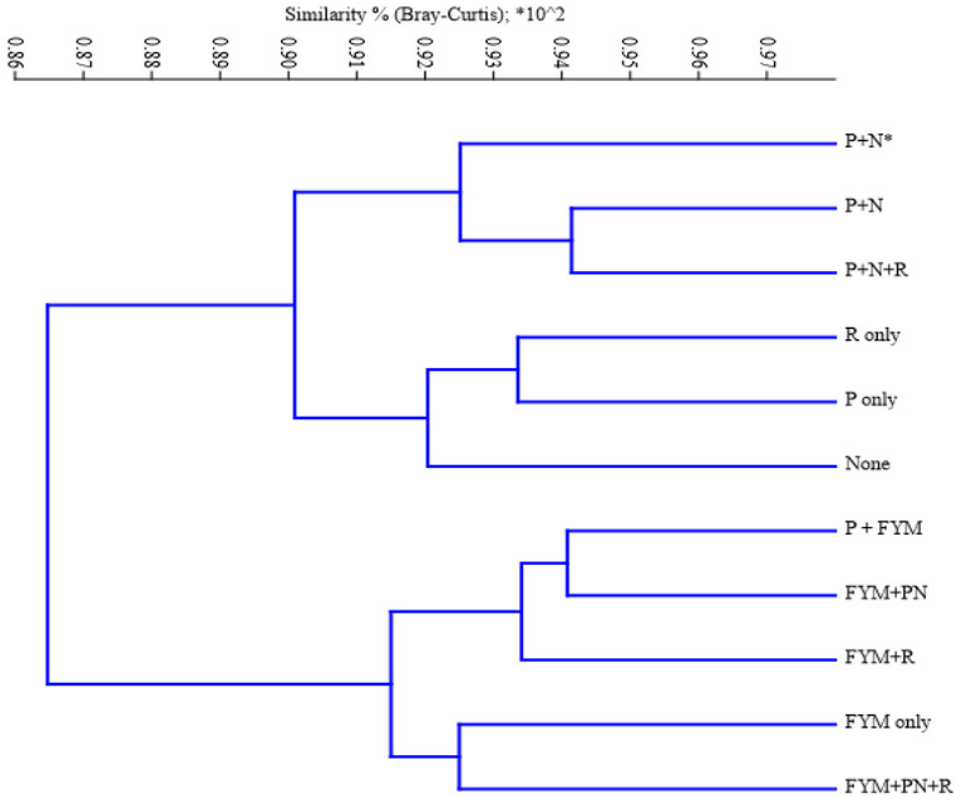

Figure 2. Bray–Curtis similarity based on hierarchical clustering of zinc solubilizing microbial species based on different soil fertility management systems in INM3 Madeya long-term trial, in the year 2019. * denotes that N was applied at 90 kg N ha^{-1}.

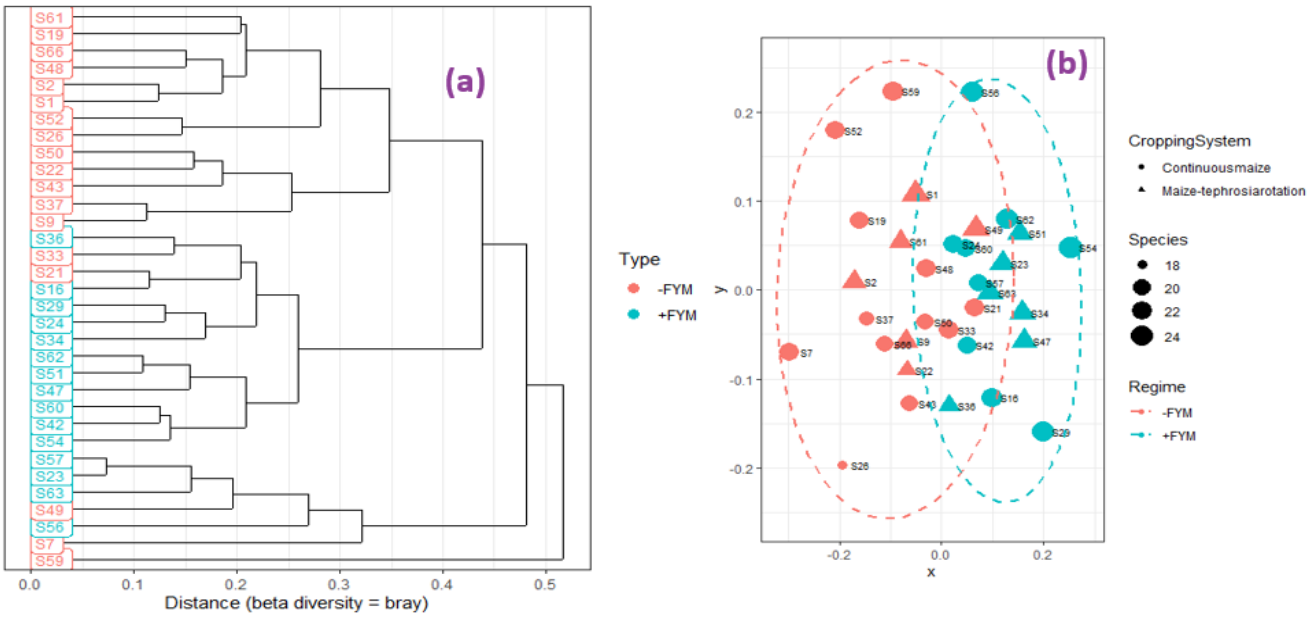

Figure 3. Dendrogram (**a**) and non-metric multidimensional scaling plot (**b**) of Zinc solubilizing microbial species (genus level) distribution in INM3 long-term trial, Western Kenya, in year 2019. S1 to S66 represent the specific samples per treatment studied.

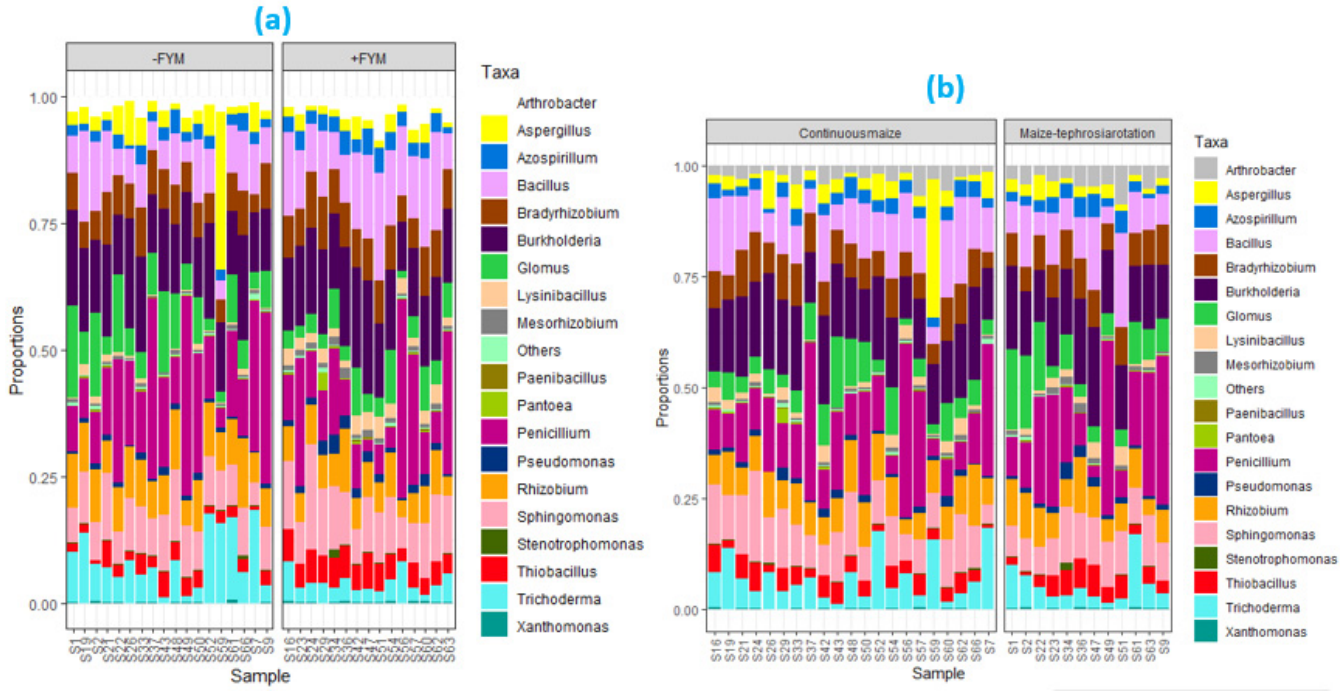

Figure 4. Proportions of ZSM species under the influence of FYM application (**a**) and cropping systems (**b**) in INM3 trial in the year 2019. S1 to S66 represent the specific samples per treatment studied.

The management practices with FYM manure addition had two times more ZSM species (with relative abundance > 0.5%) compared to those lacking FYM (Figure 5; PCA Results). *Penicillium* spp., *Glomus* spp., *Rhizobium* spp., *Sphingomonas* spp., *Aspergillus* spp. and *Trichoderma* spp. were grouped in the management systems lacking FYM, while the remaining ZSM species (i.e., *Bacillus* spp., *Azospirillum* spp., *Burkholderia* spp., *Pseudomonas* spp., *Thiobacillus* spp., *Arthrobacter* spp., *Mesorhizobium* spp., *Bradyrhizobium* spp. and *Lysinibacillus* spp.) were grouped in management practices that received either sole FYM or the combined application of FYM with other inputs (Figure 5).

Eleven (11) ZSM species comprising of *Azospirillum* spp., *Bacillus* spp., *Lysinibacillus* spp., *Mesorhizobium* spp., *Pseudomonas* spp., *Rhizobium* spp., *Stenotrophomonas* spp., *Thiobacillus* spp. and *Trichoderma* spp. were significantly ($p < 0.05$) affected by the effects of FYM application (Figure 6).

The application of inorganic N fertilizer (Figure 7) alone or in combination with FYM and crop residues influenced the proportions of specific ZSM. The proportions of *Glomus* spp., *Penicillium* spp. and *Thiobacillus* spp. were depressed under systems with nitrogen applied at 90 kgN/ha compared to no application (0 kgN ha^{-1}). The proportions of ZSM were not significantly influenced by P (data not shown). Moreover, the application of phosphorus, nitrogen and residue did not significantly affect the overall abundance and diversity of ZSM species (Table 4), although species richness and diversity were slightly higher in systems with, relative to without, P applied. Still, the application of FYM (at 4 t ha^{-1}) significantly increased ZSM abundance and diversity, and soil biochemical parameters involving SOC, total N, Zn and Fe.

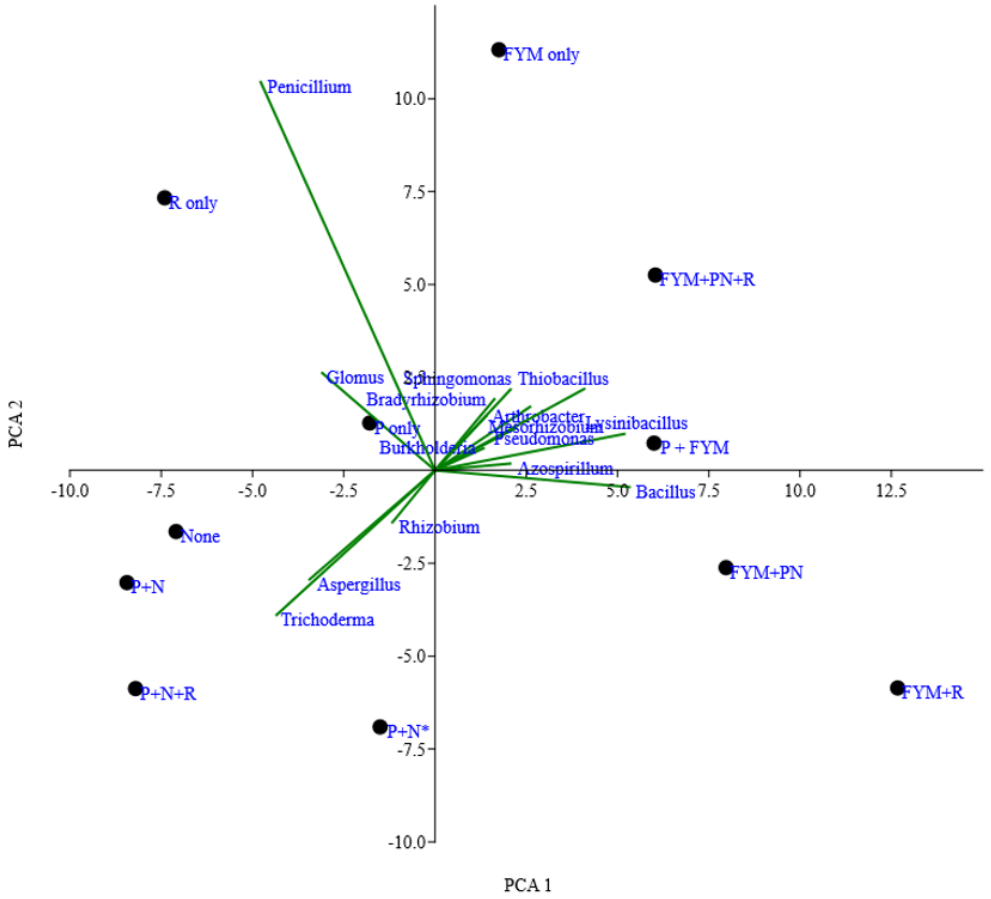

Figure 5. Effect of soil fertility management practices on zinc solubilizing microbes (at genus level with relative abundance >0.5%), as defined by principal component analysis (PCA) in INM3 Madeya long−term trial in the year 2019.

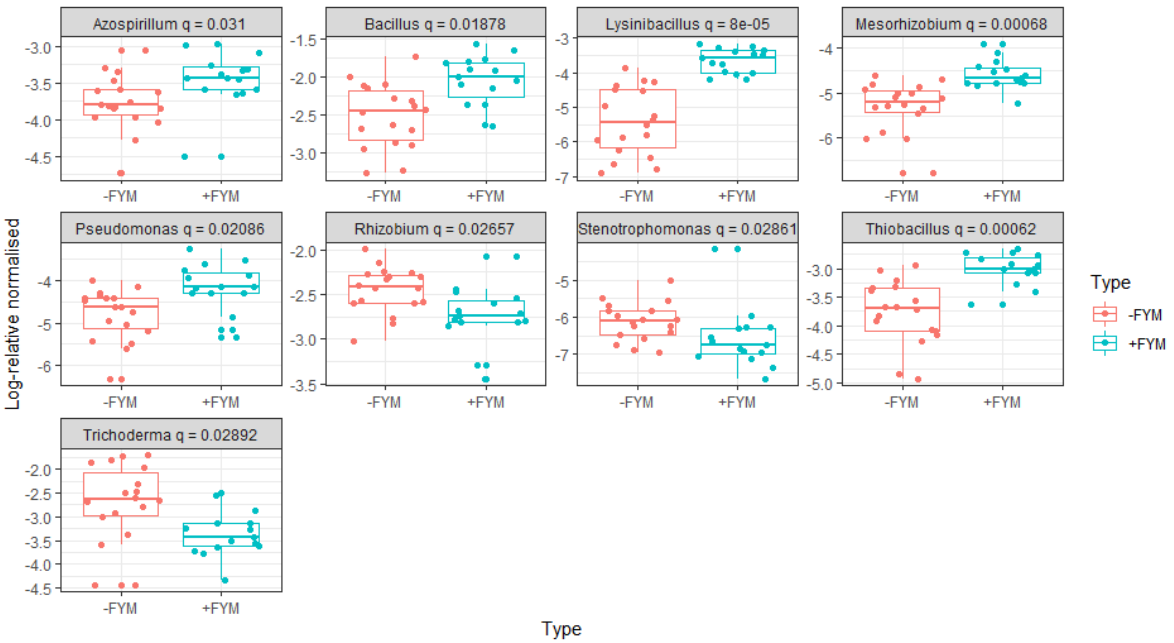

Figure 6. Zinc solubilizing microbial species (genus level) significantly influenced by management practices in INM3 Madeya long-term trial in the year 2019.

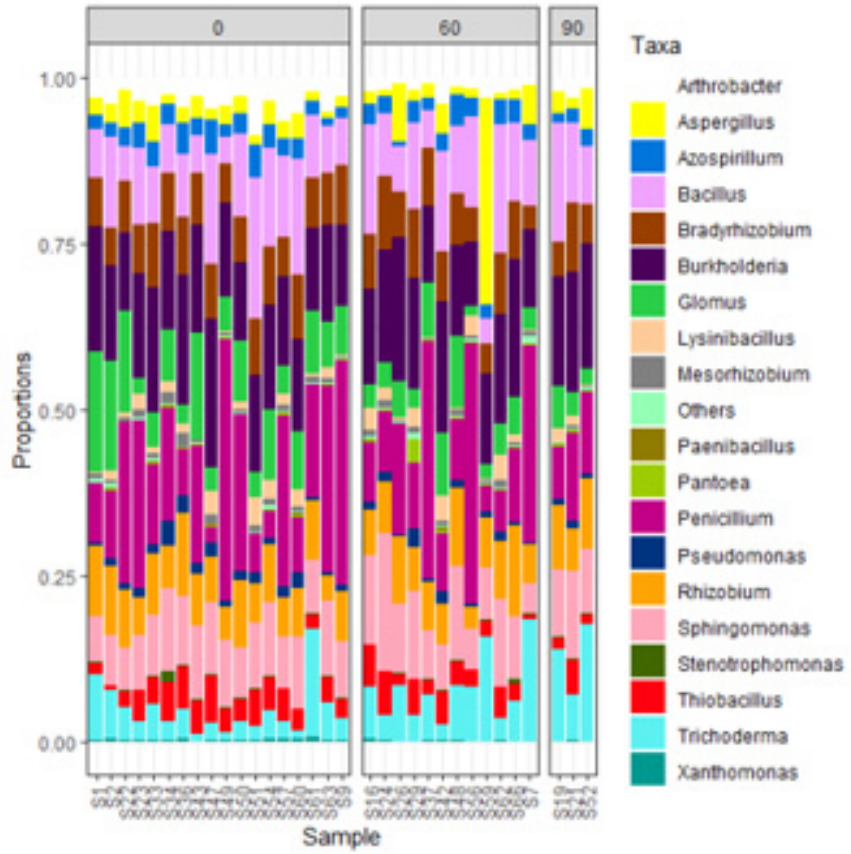

Figure 7. Proportions of ZSM species under the influence of nitrogen fertilizer application at 0, 60 and 90 kg N ha^{-1} in INM3 trial in the year 2019. S1 to S66 represent the specific samples per treatment studied.

Table 4. Zinc solubilizing microbial species richness and diversity and soil chemical parameters under different agronomic inputs in 2019.

Inputs	Diversity (Shannon)	Richness	pH	SOC (%)	N (%)	Olsen P (mg kg^{-1})	K (mg kg^{-1})	Zn (mg kg^{-1})	Fe (mg kg^{-1})
Nitrogen (kg ha^{-1})									
0	2.33 ± 0.15 ^{a}	21.2 ± 1.44 ^{a}	5.10 ± 0.26 ^{a}	1.85 ± 0.17	0.15 ± 0.02 ^{a}	31.9 ± 22.0 ^{b}	343 ± 123 ^{a}	2.12 ± 1.02 ^{a}	128 ± 15.5 ^{a}
60	2.27 ± 0.19 ^{a}	21.3 ± 2.10 ^{a}	4.80 ± 0.27 ^{a}	1.79 ± 0.18	0.14 ± 0.02 ^{a}	66.6 ± 15.8 ^{a}	251 ± 94 ^{a}	2.36 ± 1.27 ^{a}	151 ± 25.2 ^{a}
90	2.32 ± 0.07 ^{a}	21.0 ± 1.00 ^{a}	4.54 ± 0.13 ^{a}	1.75 ± 0.10	0.14 ± 0.01 ^{a}	42.5 ± 16.7 ^{ab}	162 ± 39 ^{a}	1.61 ± 0.50 ^{a}	129 ± 7.8 ^{a}
Phosphorus (kg ha^{-1})									
0	2.30 ± 0.16 ^{a}	21.1 ± 1.24 ^{a}	5.08 ± 0.26 ^{a}	1.90 ± 0.19	0.16 ± 0.02 ^{a}	18.9 ± 5.65 ^{b}	381 ± 124 ^{a}	2.07 ± 1.18 ^{a}	126 ± 14.6 ^{a}
45	2.31 ± 0.16 ^{a}	21.3 ± 1.85 ^{a}	4.86 ± 0.32 ^{a}	1.77 ± 0.15	0.14 ± 0.02 ^{a}	60.7 ± 18.0 ^{a}	243 ± 89.3 ^{a}	2.21 ± 1.04 ^{a}	143± 23.1 ^{a}
FYM (t ha^{-1})									
0	2.25 ± 0.15 ^{b}	20.44 ± 1.42 ^{b}	4.74 ± 0.21 ^{b}	1.72 ± 0.11	0.13 ± 0.01 ^{b}	46.8 ± 27.0 ^{a}	212 ± 60.5 ^{b}	1.38 ± 0.45 ^{b}	125 ± 9.8 ^{b}
4	2.38 ± 0.15 ^{a}	22.2 ± 1.37 ^{a}	5.19 ± 0.24 ^{a}	1.94 ± 0.15	0.16 ± 0.02 ^{a}	43.9 ± 23.5 ^{a}	390 ± 104 ^{a}	3.10 ± 0.82 ^{a}	150 ± 24.3 ^{a}
Residue (t ha^{-1})									
0	2.35 ± 0.12 ^{a}	21.2 ± 1.61 ^{a}	4.9 ± 0.32 ^{a}	1.79 ± 0.15	0.14 ± 0.02 ^{a}	47.1 ± 23.1 ^{a}	257 ± 103 ^{a}	2.03 ± 0.84 ^{a}	137 ± 21.1 ^{a}
2	2.23 ± 0.20 ^{a}	21.3 ± 1.76 ^{a}	5.01 ± 0.31 ^{a}	1.87 ± 0.20	0.15 ± 0.02 ^{a}	42.6 ± 29.1 ^{a}	357 ± 130 ^{a}	2.38 ± 1.42 ^{a}	136 ± 23.8 ^{a}

Values are means ± standard deviations of 3 replicate samples. Values followed by similar letters in each column, per input, are not significantly ($p \leq 0.05$) different.

3.4. Relationship between Soil Chemical Characteristics and ZSM Abundance

The ZSM microbial species richness and diversity were significantly positively correlated with SOC, pH, Ca, Mg, Zn, Fe and CEC (Table 5).

Table 5. Relationship between ZSM microbial diversity indices and soil chemical parameters in INM3 study site in 2019.

Microbial Indices	Olsen P (mg kg^{-1})	N (%)	SOC (%)	pH	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Zn (mg kg^{-1})	Fe (mg kg^{-1})	CEC (meq 100 g^{-1})
Diversity (Shannon)	−0.137	0.286	0.167	0.375 *	0.312	0.498 **	0.480 **	0.468 **	0.283	0.350 *
Species richness	0.105	0.252	0.390 *	0.272	0.300	0.382 *	0.422 *	0.348 *	0.642 **	0.514 **

*, correlation is significant at the 0.05 level (2-tailed); **, correlation is significant at the 0.01 level (2-tailed).

Among the individual ZSM species, soil total N, SOC, pH, Ca, Mg and CEC were positively correlated with *Bradyrhizobium* spp., *Arthrobacter* spp., *Thiobacillus* spp., *Pseudomonas* spp., *Azospirillum* spp., *Lysinibacillus* spp. and *Mesorhizobium* spp. (Table 6).

Canonical correspondence analysis (CCA) results showed that soil chemical parameters involving SOC, Olsen P, total N, Fe and Zn significantly affected the distribution of ZSM species (Table 7).

The distribution of seventeen (17) ZSM species (at genus level) involving, *Agrobacterium* spp., *Arthrobacter* spp., *Aspergillus* spp., *Azospirillum* spp. *Bacillus* spp., *Bradyrhizobium* spp., *Glomus* spp., *Lysinibacillus* spp., *Mesorhizobium* spp., *Pantoea* spp., *Penicillium* spp., *Pseudomonas* spp., *Rhizobium* spp., *Sphingomonas* spp., *Thiobacillus* spp., *Trichoderma* spp. and *Xanthomonas* spp. were significantly affected by the soil chemical characteristics (Figure 8; Table 7). *Lysinibacillus* spp. was strongly correlated with soil available Zn, and *Sphingomonas* spp. and Serratia spp. with Olsen P, while *Thiobacillus* spp. was strongly correlated with S and SOC (Figure 8).

Table 6. Relationship between soil chemical characteristics and ZSM abundance (counts) in INM3 Madeya in year 2019.

Variables	*Burkholderia* spp.	*Bacillus* spp.	*Trichoderma* spp.	*Rhizobium* spp.	*Sphingomonas* spp.	*Bradyrhizobium* spp.	*Glomus* spp.	*Aspergillus* spp.	*Arthrobacter* spp.	*Thiobacillus* spp.	*Pseudomonas* spp.	*Azospirillum* spp.	*Lysinibacillus* spp.	*Mesorhizobium* spp.	*Paenibacillus* spp.	*Pantoea* spp.	*Xanthomonas* spp.	*Staphylococcus* spp.	*Mucor* spp.	*Serratia* spp.	*Agrobacterium* spp.	*Micrococcus* spp.	*Sporosarcina* spp.
P §§	0.01	0.17	0.37*	0.24	0.02	−0.06	−0.23	0.26	−0.33	−0.19	−0.24	0.08	0.09	−0.24	0.19	0.08	−0.33	0.25	0.01	0.25	−0.08	−0.02	0.10
N (%)	0.41 *	0.37 *	−0.35 *	−0.21	0.41 *	0.47 **	−0.30	−0.19	0.64 **	0.77 **	0.57 **	0.52 **	0.49 **	0.78 **	0.17	−0.02	0.46 **	0.12	−0.30	−0.17	0.35 *	0.16	0.07
SOC (%)	0.38 *	0.37 *	−0.31	−0.44*	0.60 **	0.57 **	−0.18	−0.12	0.47 **	0.74 **	0.54 **	0.45*	0.52 **	0.71 **	0.05	−0.03	0.21	0.18	−0.25	−0.12	0.37 *	0.30	0.20
pH	0.11	0.30	−0.53 **	−0.35*	0.24	0.48 **	−0.04	−0.15	0.65 **	0.65 **	0.60 **	0.51 **	0.47 **	0.65 **	0.06	0.03	0.42 *	0.14	−0.42 *	−0.09	0.37 *	0.19	−0.08
K §§	0.17	0.33	−0.48 **	−0.30	0.23	0.44 *	−0.08	−0.27	0.46 **	0.57 **	0.55 **	0.49 **	0.54 **	0.69 **	−0.09	0.09	0.22	−0.01	−0.29	−0.24	0.48 **	0.22	−0.04
Ca §§	0.20	0.52 **	−0.42 *	−0.33	0.24	0.39 *	−0.27	−0.14	0.60 **	0.71 **	0.66 **	0.57 **	0.61 **	0.58 **	0.29	0.12	0.50 **	0.18	−0.45 **	0.07	0.32	0.16	0.19
Mg §§	0.24	0.50 **	−0.45 **	−0.37*	0.41 *	0.55 **	−0.30	−0.16	0.42*	0.71 **	0.60 **	0.53 **	0.66 **	0.60 **	0.14	0.11	0.30	0.21	−0.41 *	0.06	0.28	0.23	0.19
Mn §§	0.34 *	−0.19	0.40 *	0.62 **	0.18	−0.10	−0.22	0.49 **	0.17	−0.03	0.06	0.23	−0.34	0.22	−0.07	−0.13	−0.11	0.26	0.28	−0.21	−0.11	0.21	−0.36 *
S §§	−0.04	0.30	−0.12	0.07	−0.12	−0.08	−0.40 *	−0.05	−0.08	0.05	0.04	0.06	0.17	−0.19	0.40 *	0.03	0.05	0.08	−0.26	0.37 *	−0.09	−0.15	0.06
Cu §§	0.09	0.37 *	−0.30	−0.09	0.41 *	0.21	−0.21	−0.01	0.26	0.45 **	0.51 **	0.31	0.45 **	0.30	0.23	−0.02	0.05	0.16	−0.15	−0.09	0.18	0.46 **	0.21
B §§	0.15	0.28	−0.26	−0.31	0.11	0.14	−0.2	−0.10	0.62 **	0.47 **	0.47 **	0.43 *	0.26	0.60 **	0.27	−0.12	0.38*	0.14	−0.31	−0.12	0.14	0.11	−0.03
Zn §§	0.29	0.48 **	−0.32	−0.20	0.52 **	0.59 **	−0.43 *	−0.21	0.28	0.71 **	0.48 **	0.46 **	0.65 **	0.59 **	0.11	0.21	0.30	0.13	−0.34	0.11	0.21	0.14	0.23
Fe §§	0.29	0.37 *	−0.05	−0.16	0.61 **	0.55 **	−0.21	−0.01	−0.08	0.52 **	0.33	0.25	0.54 **	0.33	0.19	0.50 **	0.05	0.14	−0.26	0.02	0.34	0.10	0.36 *
Na §§	0.25	−0.02	−0.23	−0.25	0.45 **	0.27	−0.10	0.11	0.02	0.37*	0.33	0.01	0.10	0.04	0.05	0.09	−0.03	0.05	−0.30	0.16	0.11	0.15	0.22
EC ¥	0.21	0.32	0.10	0.01	0.23	0.03	−0.33	0.01	−0.02	0.23	−0.05	−0.03	0.36 *	0.02	0.48 **	−0.08	0.14	0.03	−0.02	0.21	0.07	−0.05	0.41 *
CEC #	0.41 *	0.51 **	−0.18	−0.19	0.54 **	0.51 **	−0.42 *	0.18	0.50 **	0.82 **	0.64 **	0.57 **	0.63 **	0.62 **	0.30	0.15	0.37 *	0.37 *	−0.34	0.01	0.25	0.23	0.32

Only ZSM significantly correlating with at least one chemical variable presented. SOC = soil organic carbon, EC = electrical conductivity; CEC = cation exchange capacity; §§ = mg kg^{-1}; ¥ = uS/cm; # represents meq/100 g; * represents significant correlation at the 0.05 level; ** represents significant correlation at the 0.01 level (2-tailed).

Table 7. Bi-plot CCA analysis scores for constraining zinc solubilizing microbial species and soil chemical properties in INM3 site in year 2019.

	CCA1	CCA2	R^2	*p*-Value
Zinc solubilizing microbial genera				
Agrobacterium	0.91	0.42	0.27	0.02
Arthrobacter	0.64	0.76	0.66	0.001
Aspergillus	0.98	−0.21	0.51	0.001
Azospirillum	0.96	0.26	0.39	0.001
Bacillus	1.00	0.03	0.57	0.001
Bradyrhizobium	0.90	−0.43	0.42	0.001
Burkholderia	1.00	−0.01	0.03	0.67
Enterobacter	1.00	0.10	0.02	0.73
Glomus	0.23	−0.97	0.19	0.05
Gluconacetobacter	0.62	0.79	0.02	0.71
Klebsiella	0.99	0.12	0.02	0.68
Lysinibacillus	1.00	−0.05	0.76	0.001
Mesorhizobium	0.92	0.40	0.33	0.01
Paenibacillus	0.81	0.58	0.07	0.38
Pantoea	0.33	−0.94	0.69	0.004
Penicillium	0.24	0.97	0.84	0.001
Pseudomonas	0.98	−0.22	0.32	0.03
Rhizobium	−1.00	−0.04	0.38	0.002
Serratia	−0.09	−1.00	0.02	0.64
Sphingomonas	0.75	−0.66	0.26	0.03
Staphylococcus	0.67	0.74	0.06	0.47
Stenotrophomonas	−0.31	0.95	0.01	0.84
Thiobacillus	1.00	0.04	0.59	0.001
Trichoderma	1.00	−0.01	0.67	0.001
Xanthomonas	0.71	0.70	0.22	0.05
Soil chemical properties				
Olsen P	−0.11	−0.41		0.04
N	0.71	0.45		0.001
Zn	0.76	−0.17		0.02
pH	0.77	0.44		0.13
SOC	0.38	−0.06		0.05
S	0.4	0.03		0.22
B	0.55	0.62		0.69
Fe	0.57	−0.76		0.03
Cu	0.31	0.09		0.56

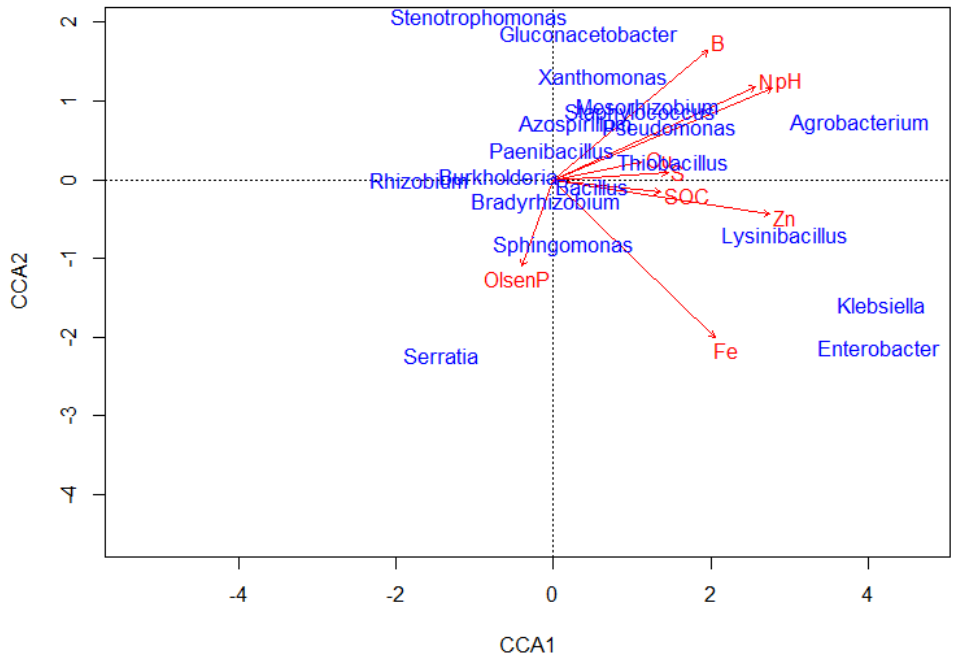

Figure 8. Canonical correspondence analysis of the relationship between soil chemical characteristics and zinc solubilizing microbial species distribution in INM3 site in year 2019.

4. Discussion

The ability of soil microbes to solubilize zinc is of interest since it can enhance the availability of zinc for plant growth and ultimately stimulate its deposition in edible plant tissues [43,44]. Nitrogen, phosphorus, potassium and zinc are among the major limiting nutrients for sustainable crop productivity [45], whose availability may be influenced by both environmental conditions and/or agronomic management practices. Some of these essential plant nutrients may be susceptible to leaching, slow solubility, poor mobility and fixation in insoluble compounds [46,47], diminishing their availability for crop uptake; hence, leading to deficiencies that not only affect crop production but also nutritional quality.

4.1. Influence of Soil Chemical Properties on Zinc Solubilizing Microbial Species

In this study, we hypothesized that the soil chemical and biological properties are critical in determining soil microbial species abundance, distribution and diversity, and thus, would influence the zinc solubilizing microbial species abundance and distribution. The results from correlations and CCA showed that the soil chemical and biological parameters were relevant to the distribution and abundance of the zinc solubilizing microbes. These results corroborate recent findings on the effects of different soil nutrients on related soil microbial properties at the same site [37,48,49] and elsewhere in Australia [50] and China [51]. The significant effects of SOC, macronutrients (Olsen P and total N) and micronutrients (Fe and Zn) on zinc solubilizing microbial species abundance and distribution indicate the important role of nutrition in driving the microbial community distribution and abundance. Previous reports [42,48,52,53] linked certain soil microbial parameters to nutrient enrichment and soil organic carbon accumulation, providing food and energy for microbial growth and development. At various scales, the soil microbial community often displays heterogeneous distribution, and this is majorly due to the influences of prevailing soil conditions involving soil fertility (nutrient supply) status, and physicochemical and biological properties. In addition to microbial distribution, the dynamics of microbial abundance are also influenced by the soil fertility status and physicochemical conditions [37]. Thus, in most instances, the microbial abundance can increase with increasing nutrient availability (i.e., soil fertility) and decrease with declining nutrient availability [42], although the reverse may be true in some instances involving specific microbial groups. This explains the significant positive correlations observed between numerous soil chemical parameters and zinc solubilizing microbial species.

We also observed a slight negative effect of soil N (i.e., when N was applied at 90 kgN ha^{-1}) on the proportions of certain ZSM populations (*Glomus, Penicillium* and *Thiobacillus*), and this indicates either limited access to soil available N or low N demand by some of these ZSMs. Nitrogen occurs in soils in mostly complex organic forms and this limits its access by microbes that lack saprotrophic nutrition capabilities such as in Glomus [54]. In addition, the few observed ZSM species that negatively correlated with some soil characteristics suggest the likelihood of resource (nutrient) competition existing between them and the other species that had positive correlations. *Thiobacillus* are not only zinc solubilizers but also sulfur oxidizers [55], thus explaining their positive correlation with soil S. SOC provides food that releases vital energy required by microbes for growth and development. The influences of different soil chemical (and biological) parameters on soil microbial structure have previously been observed, with some increasing, decreasing or having no change in response [37,42].

There were no major shifts in microbial species proportions under the two cropping systems (i.e., maize and Tephrosia rotation and continuous maize systems), and this demonstrates the high similarity of such systems in terms of ZSM colonization. This also points to the possibility of small-scale soil microbial heterogeneity within the two cropping systems, perhaps contributed to by the sampling location (plot level identity). In a previous study, ref. [42] reported that the abundance of the microbial groups tested depended on the sampling location, with small-scale biotic and abiotic heterogeneity contributing a great deal in influencing microbial species coexistence. Similarly, in a study conducted in long-term

no-till systems in Tennessee, crop rotation sequence did not influence bacterial richness, diversity and community structure [56]. This also corroborates previous observations in a study conducted on a Brazilian Oxisol where rotation with different crops did not influence related microbial parameters [57]. However, the findings contradict previous observations by [37] where related microbial functional groups associated with phosphorus solubilization were significantly affected by cropping systems perhaps due to the systems that were under comparison. Study [37] compared maize soybean intercropping versus rotation under conservation tillage as opposed to the current study that compared maize–Tephrosia rotation versus sole maize under a continuously tilled integrated soil fertility management system.

4.2. Stimulation of Zinc Solubilizing Microbial Species with FYM Application

Regenerative agriculture practices involving the incorporation of organic matter (farmyard manure application and residue retention) among others [58], can strongly influence the soil physicochemical parameters and biological properties and enhance soil fertility by stimulating microbial abundance and nutrient cycling and regulating SOC availability [37]. The application of organic matter (i.e., FYM) has been shown to improve soil physiochemical conditions [59] alongside stimulating a positive microbial link between nutrient availability [60], the regulation of SOC and moisture. In our study, the application of farmyard manure not only influenced the distribution but also increased the abundance and diversity of several zinc solubilizing microbial species in the INM3 site.

The HCA revealed a systematic grouping, where the ZSMs were clustered in two major clades, depicting the effect of either the application of (or no addition of) organic matter (FYM) on the ZSM. FYM is important in improving nutrient availability and soil pH, moderating temperature, moisture retention, and enhancing SOC availability [31,32,34], and this creates favorable and enabling conditions that stimulate microbial proliferation and activities [33,35]. Some of the ZSM species are copiotrophic, preferring nutrient- and carbon-rich environments [61,62], and the addition of FYM could enhance soil nutrients' and carbon availability, thereby stimulating the copiotrophic ZSM species. However, the clustering of the treatments without FYM application (but with other inputs) in a different major clade depicts that not all ZSM species are copiotrophic, but rather, some would likely prefer certain environmental conditions not influenced by the availability of FYM. Moreover, the differences in the similarity indices of different management systems in different subclades (subclusters, Figure 4) under each major clade perhaps depict that the ZSM species in the different management system pairs were not quite homogeneous, and this echoes the previous suggestion by [63].

The clustering of the ZSM species within a particular management system (i.e., with either the application or no application of farmyard manure) observed in this study demonstrates the strong similarity of microbial groups within each of those management systems. The slight shift in clustering of the microbial species in one management system distinct from the other management system as observed indicates heterogeneity between the two particular systems. This heterogeneity can be attributed to the effects of the specific management systems on soil physiochemical and biological conditions that would, consequently and concomitantly, either directly or indirectly influence the different microbial parameters. This involves influences on soil pH, SOC, moisture, temperature, nutrient availability/fertility and numerous biological characteristics involving the effects on certain microbial structures that can also alter microbial community composition in a given system. These assertions strongly relate to the observed ZSM microbial species' responses to the application of FYM. In this study, as well as the ZSM microbial species being distinctly grouped with either the application or no application of FYM, the systems with FYM added had more ZSM species abundance with the majority being significantly elevated following the addition of FYM. Yet, ZSM species' richness and diversity (Shannon), SOC and soil pH and nutrient supply (macro- and micronutrients) were elevated under systems with FYM application compared to those lacking FYM, implying the beneficial influences of

FYM on soil biochemical conditions. The findings from this study further showed that the management systems without FYM application had zinc concentrations ranges between 1.0 and 1.84 mg kg^{-1}, and these ranges were below the critical soil zinc deficiency threshold (0.6–2 mg kg^{-1}; [5]). However, all the systems where FYM was added solely or in combination with other nutrients had zinc concentration ranges (2.61–3.57 mg kg^{-1}) above the critical deficiency threshold of 0.6–2.0 mg kg^{-1}, indicating the likelihood of FYM increasing zinc availability, perhaps through the enhancement of zinc solubilizer abundance.

Among other observations, the increase in ZSM species richness (abundance) and diversity following the addition of FYM relative to no application could also be attributed to the effect of FYM in increasing SOC and nutrient availability, and the creation of a microclimate that would favor microbial proliferation, as observed in the known copiotrophic ZSM genera involving *Pseudomonas* spp., *Bacillus* spp. and *Rhizobium* spp. [43] that thrive in nutrient rich environments. Consistent with the results of this study, previous papers [64,65] have reported increased microbial species abundance and diversity following farmyard manure application.

The significant positive correlation between ZSM species richness/diversity with soil pH, zinc and other nutrients denotes microbial nutrient (especially Zn) demand, and this may culminate in a variation in the intensity of solubilization activities. Moreover, the significant correlation between available soil zinc content with ZSM abundance also points to increased microbial solubilization and/or the mineralization of different pools of zinc. In the study site, available soil zinc concentrations ranged between 1.00 mg/kg and 3.57 mg/kg, which is within the critical soil zinc deficiency threshold (0.6–2.0 mg/kg, [5]), implying that zinc deficiency is an issue of concern in this site. However, it is interesting to point out that the highest values of zinc (above the critical threshold of 2.0 mg/kg) were only recorded in the systems with either the sole application of FYM or the combined application of FYM with other inputs (full fertilization), with the ZSM species abundance significantly correlating with zinc availability. This denotes the potential of FYM application in not only increasing zinc solubilizing microbial abundance, but also the available zinc concentrations beyond the deficiency threshold limits by stimulating ZSM solubilization activities. The positive capacity of manure to provide multiple nutrients and stimulate biological parameters was recently acknowledged [33–35]. The increase in soil zinc concentrations (above critical thresholds) and also ZSM abundance and diversity following the application of FYM, either alone or in combination with other nutrients, is an indication that FYM, upon mineralization, can significantly influence micronutrient availability, and that combining FYM with other inorganic nutrients at appropriate rates can further provide more environmental benefits by increasing zinc availability but also stimulating zinc solubilizing microbial species abundance, diversity and activities.

Some of the ZSM species also perform different functions other than zinc solubilization in the soil, and could be affected by other parameters directly linked to FYM application. Recent studies have shown that some of the ZSM species (i.e., *Penicillium* spp., *Glomus* spp., *Rhizobium* spp., *Sphingomonas* spp., *Aspergillus* spp. and *Trichoderma* spp.) that were distinctively grouped in the management practices lacking FYM are also functional phosphorus solubilizing microbes that are negatively affected by increasing P availability [37,66,67]. The addition of FYM could stimulate soil P availability upon decomposition and mineralization, thereby suppressing some of the phosphorus-solubilization-linked strains of ZSM species. In the study, the application of FYM significantly increased pH relative to no application, and this contributed to 31.4% increase in soil P (Table 2), which could influence the P-solubilization-associated ZSM species.

Credible information on the relationship between zinc solubilizers' abundance and diversity with plant nutrition (zinc uptake) is largely missing and requires further research. In addition, it is still unclear what the crop yields' trends/patterns would be, relevant to the quantities of Zn available from microbial solubilization compared to the application of Zn-based fertilizers, and whether the quantities of Zn solubilized would be dependable for sustainable crop yields. Still, the quantities of Zn that can be potentially solubilized

by these microbes from the different pools lies widely unknown. Moreover, a dearth of knowledge still exists on whether the nutrient (Zn) quantities solubilized by the microbes can be in measures that could sustainably offset the soil deficiency thresholds, and the right combination of systems to optimize the realization of microbial-driven nutrient (Zn) solubilization. Furthermore, in addition to Zn-based fertilizers being costly, there is scanty knowledge on the potential economic benefits that can accrue from microbial-derived nutrient (Zn) mineralization versus the use of inorganic zinc-based fertilizers in enhancing crop production.

5. Conclusions

This study revealed that the application of FYM increases ZSM abundance and diversity. This is important in the quest for sustainable agricultural production by enhancing the provision and availability of multiple soil nutrients for balanced plant (and human) nutrition. As well as macronutrients, the application of FYM improved soil zinc availability, attributable to increased microbial (especially the ZSM) abundance and activities. The study also demonstrated that the application of inorganic N fertilizer, either alone or in combination with FYM and crop residues, influenced the proportions of specific ZSM. Furthermore, the improvement in key soil chemical parameters with the application of FYM stimulated ZSM species richness and abundance. These positive ZSM responses with manure application indicate the potential for biologically mediated soil Zn enrichment as an alternative to Zn-based fertilizer application, to enhance sustainable, ecofriendly and cost-effective Zn bioavailability and supplementation for plants.

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