

Spatial Distribution of Trophic Groups of Amoebae Around the Root Zone of *Zea Mays* Mycorrhizal With *Rhizophagus Intraradices* Grown in Microcosms

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Abstract

Fitness and productivity of most terrestrial plants depend on early associations with arbuscular mycorrhizal fungi and mutualistic bacteria. Plants select most of the microbial communities cohabiting their roots and mycorrhizosphere, attracting also all types of microbial predators. Naked amoebae are among the most voracious predators inflicting significant changes in soils bacterial and fungal populations. We evaluated how roots of *Zea mays* with or without *Rhizophagus intraradices* mycorrhizosphere (AMF) influence trophic groups of amoebae, along vertical (3, 6, and 9-cm) and horizontal soil distribution (roots and free-root compartments) grown in microcosms after, 20 days. Amoebae community in Non-AMF showed a high species richness in the root zone at 3 to 6-cm depth, and at the two free-root compartment away from plants. Conversely, AMF and mycelium zones modified the amoeba community at 6 to 9-cm depth, recording higher diversity of trophic groups than unplanted soil compartments. The highest bacterivorous diversity was found at the closer compartment to AMF roots, but fungivorous amoebae was not recorded. Amoebae feeding preferences were similar in both AMF and Non-AMF microcosms in where bacterivorous amoebae were dominant, while protozoa-eating amoebae were more frequent at the mycelium compartments. Rare amoebae species were found in AMF microcosms in comparison to those recorded from Non-AMF and unplanted microcosms.

Introduction

Productivity ecosystem and agrosystems [1] are based on interactions among plants-bacteria-fungi. Arbuscular mycorrhizal fungi (AMF) are determinant for soil nutrient mobilization, and plant nutrient uptake and productivity [2–4]. Once AMF are successfully established, metabolic activity and extraradical-mycelium exudation create specific microenvironments that modify the microbial community composition and their physiological activities (mycorrhizosphere effect [5, 6]). This functional zone has two main components: 1) rhizosphere with AMF-mycelium, and 2) hyphosphere integrated by AMF-hyphae network [7].

Fungal exudates from mycorrhizosphere generate hotspots for microbial communities with higher functional diversity [8–10]. Similarly, AMF-hyphosphere increases plant nutrient uptake from distant soil matrixes and also allows specific bacterial activities around hyphae [7, 8, 10] and spores [11], that enhance spore germination, hyphal growth, and root colonization [12, 13]. Hyphal exudates of some AMF species increase the abundance of Oxalobacteraceae, Streptomycetes and Firmicutes [14, 9, 10], around and inside AMF mycelium [10]. Furthermore, hyphae-associated bacteria may provide protection against fungal grazers, promote root colonization [15, 16]. Conversely Acidobacteria, Bacteroidetes, Firmicutes and Proteobacteria species can suppress mycorrhizal development [17] as well as microorganisms that cause plant diseases [18–21].

Plant and fungal exudations attract predators which enhance nutrient cycling around root systems and mycorrhizosphere [22]. Protozoa locate their preys through recognition of chemical signals produced by plants and microorganisms [23]. Thus, prey selection and consumption are based on bacterial exudates, sizes, and palatability [23–25].

Amoeba are main bacterial predators from soil [22, 23, 26–28], and almost every amoebae species temporarily survive by feeding on bacteria. However, amoebae feed on different kind of organisms, and based on their feeding preferences, can be grouped as bacterivorous, fungivorous, yeast-eaters, algae-eaters, protist-eaters, and omnivorous. This interactions predator-preys are part of Microbial Loop phenomenon that boistimulate the plant growth. [29]. Amoebae community develops within a short distance of plant-microbial hotspots. However, how trophic groups of amoebae influence productivity and trophic web is still unknown in general. Thus, we addressed the question about how AMF (*Rhizophagus intraradices*) colonizing roots of *Zea mays* may modify the structure of amoebae trophic groups along vertical distribution (3, 6, and 9-cm depths) after 20 days? Then, we aimed to determine the short-term effects of AMF and roots of *Z. mays* on amoebae community in a microcosms study.

Material And Methods

Soil volume determination

Twenty-four composite sandy-soil samples were taken from roots zone of non-mycorrhizal pots planted with *Zea mays* at V8 stage. Each sample (1 g dry weight), was homogenized in 10 mL of sterile sandy-soil-extract solution (1:10 v/v) by shaking twice at 2000 rpm during 30 s. After allowing soil-particles sedimentation (30 min), supernatant was poured on soil-extract agar. Then Petri dish tilted at 10 degrees for 2 h to facilitate trophozoites attaching on agar surface. Excess water was removed with sterile Pasteur pipette carried out into sterile conditions [30]. Petri dishes were incubated at 28°C and reviewed daily for 3 weeks for amoebae identification (Table S1).

Amoebae Observation

Fresh preparations were used to visualize amoebae. Trophozoites were recuperated by suspension through scraping on agar with loopful in 300 µL sterile distilled water. This suspension was recovered with a sterile Pasteur pipette and poured on slide and spread with coverslip. Preparations were allowed to settle down in humid chamber during 3 h before observation with BX51 phase contrast microscope at 20X, 40X and 100X magnifications. Amoebae videos were recorded for id corroboration (40X). Morphological identification was based on specialized keys [1–6] as well as specialized papers.

Soil-extract (Stock Solution)

Sandy-soil suspensions (300 g/1 L distilled water) were placed in water bath at 60° C for 3 h and filtered throughout Whatman No.2 filter paper; filtrate was sterilized and stored at 4°C until utilization. Solid medium was prepared by dissolving bacteriological-agar 15 g/L in soil-extract working solution (diluting 1:5 v/v, soil-extract/water). Soil-extract solution used to homogenize soil subsamples was prepared at 1:10 v/v [30].

Microcosms and experimental settings

Microcosms (6 unities) were fabricated as rectangular acrylic containers of 18 cm length x 12 cm wide x 10 cm height. Microcosms were divided in three compartments filled up with 350 g of sterilized sand and separated by two stainless-steel meshes, pore size: 44 µm, this pores allows AMF-hyphae passing to adjacent compartments while restricting roots passing (Fig S1).

Microbial inoculum (slurry)

Ninety grams of sandy soil from non-mycorrhizal roots of *Zea mays* (V8 stage) were homogenized by shaking (5-pulses for 1 min at 2000 rpm) in 810 mL sterile soil-extract (1:10 v/v). Solution was vacuum filtered, with a Kitasato flask and Buchner funnel with Whatman paper Grade-4 (20-25 µm) to allow protists and bacteria passage. Slurry was shaken with a magnetic stirrer at low velocity to keep microorganisms suspended until inoculation [30].

Microcosms inoculation

Microorganisms were inoculated by adding 50 mL of slurry in each compartment from all microcosms, which were placed in growth chamber [12 h photoperiod; 400 W Na-vapor lamps, 24°C (maximum) and 12°C (minimum)] for 20 days. Microcosms were kept at 60% water-holding capacity by watering every two days with sterilized tap water (Fig S2).

Experimental treatments

Inoculated microcosms were divided in to set three treatments by duplicate: a) unplanted-microcosms (Control=C); b) non-inoculated 8-days old *Zea mays* seedlings (N/AMF) transplanted at compartment 1 (CI), and c) *Z. mays* seedlings inoculated with *R. intraradices* (AMF) [46]. Plant inoculation was performed with 150 AMF-spores/mL⁻¹ (10 g-inoculum). Mycorrhizal-inoculum was provided by Corporativo de Desarrollo Sustentable S.A. de C.V., Morelia, Michoacan (Mexico). Compartments 2 and 3 (CII and CIII) were also inoculated with AMF-inoculum.

Previously, seeds were disinfested by washing them with 10% NaClO for 5 min, rinsed and germinated in Petri dishes with autoclaved sand (121° C for 3 h) kept at room temperature for 8 days. All microcosms were kept growth chamber conditions and kept soil at 60% of water-holding capacity for 20 days (Fig S2).

Sampling-soil from microcosms

Sampling-soil was achieved with PVC tubes (corer) with 10-cm long and 1.5-cm diameter; by quadruplicate for every compartment taking carefully a square of 3-cm between sampling-corer points. Corer was sown to 9-cm depth, provided 9 g of sand-sample allowing to obtain 3 g of sandy-soil from 3, 6 and 9-cm depths. Each 3 g sample was processes independently, obtaining 108 g of sandy-soil in 34 samples from each microcosms (Fig S2).

Amoebae trophic preferences

Amoebae feeding preferences were grouped into 6 categories: bacterivorous (B), fungivorous (F), algal-eaters (A), protozoa-eaters (P), omnivorous (O), and non-determined trophic group (ND) following the reports provided in the literature (Tables S1, S4).

Morphotypes and Most Probable Number (MPN) of amoebae, and mycorrhizal parameters

MPN of amoebae morphotypes from every subsample was determined with 1 g sandy soil homogenized in sterile soil-extract (10 mL: 1:10 v/v). Sequential serial dilutions from 10⁻¹ to 10⁻⁶, were dispensed in 24-well microplates. Aliquot of 100 µL from each dilution was dispensed in their corresponding row by quadruplicate (Fig S3). Morphotypes (I-XII, Fig. 1) were registered in every microplate after inverted microscope examination. MPN calculation was performed with Thomas formula [30].

$$\text{MPN} = \frac{\text{Number of positive tubes}}{\sqrt{(\text{Number of ml of sample of negative tubes}) \times (\text{Number of ml of all tubes})}} \times 100$$

Determination of AMF colonization in roots of *Zea mays* was done in accordance too Phillips and Hayman procedure [31], and total length of AMF mycelium was performed by Tennant method [32].

Statically Analysis

Amoebae species richness were utilized for estimating gamma diversity: 1) number of species observed, 2) second-order jackknife estimate, 3) Chao2 estimate, classic form, 4) Chao2 estimate bias corrected form (Table S2). Rarefaction analysis indicated 15 g as minimum volume to make comparison between communities from treatments and slurry preparation, with 70% of amoebae species recognized in soil composite sample from V8 maize-microcosms (Table S1, Fig S4). Sørensen similitude index was calculated by PC Ord© software ver. 7 for Windows© (dendrograms were calculated by centroid and Bray-Curtis technique) [33]. Trophic diversity was calculated by Shannon index: $H' = -\sum p_i \ln p_i$; where p_i is the proportion of i -th species inside a trophic group [34]. Simpson diversity index was calculated by equation: $D = 1 / \sum (n_i/N)^2$ where n_i is species richness in each trophic group i , and N is species number total [35]. Data obtained were compared by Chi-squared test [36]. β diversity was calculated by three algorithms: 1) Whittaker measures (1972) $\beta_w = s/\bar{\alpha} - 1$, where s species number recorded is total in each system, and $\bar{\alpha}$ is average species number found within community samples. 2) Cody (1975) or biotic change measure inside gradient habitat, $\beta_c = g(H) + l(H)/2$, where $g(H)$ were species gained and $l(H)$ were species losses. 3) Cody (1993) $\beta_{co} = 1 - C(T_1 + T_2) / 2TT_2$, where $T = \sum e_i = \sum a_j$; C = species in common between two censuses; T_i = total number of species in census i 4) β_T ("beta turnover", Wilson and Shmida, 1984), combines species turnover by gaining (g) and losing (l) species along gradient, $\beta_T = g(H) + l(H) / 2 \bar{\alpha}$ [37]. Pearson's correlation (ρ) was calculated between 1) MPN with mycelium length, and 2) amoebae species richness with mycelium length (Excel program Windows©).

Results

Amoebae MPN

Control showed in total, 3,243 individuals per g^{-1} soil-dry-weight ($i g^{-1}$ sdw), and CI showed 1023 $i g^{-1}$ sdw at 9-cm depth (Table 1). Morphotypes VI (Amoebidae: 225 $i g^{-1}$ sdw) and VII (Leptomyxidae: 151 $i g^{-1}$ sdw) were dominant at 9-cm depth from CI. Morphotype II (*Platyamoeba*: 151 $i g^{-1}$ sdw) was dominant at 9-cm depth in CII. Morphotypes VII (191 $i g^{-1}$ sdw) and III (*Hartmannella*: 112 $i g^{-1}$ sdw) were dominant for CIII (Fig. 2a; χ^2 , $P = 0.05$; Table S3).

Table 1

Species richness (SR) and most probable number (MPN) of soil free-living amoebae in Unplanted (control) sandy soil (A), N/AMF planted sandy soil (B), and AMF-planted sandy soil (C). Columns (C-I, C-II and C-III) corresponding to compartments of the mycorrhizospheric-box. The species richness is the account of different species recorded in the three compartments rather than the sum of each compartment. The total number of species richness by layer (SRT) and the total MPN by layer I are also included. Row headings: 3-cm, 6-cm and 9-cm are the sampling depths. Row heading: Total by MPN is the sum of quantities reported in the 3 depths (3, 6 and 9-cm)

	Control						N/AMF						AMF					
	CI		CII		CIII		CI		CII		CIII		CI		CII		CIII	
	SR	MPN	SR	MPN	SR	MPN	SR	MPN	SR	MPN	SR	MPN	SR	MPN	SR	MPN	SR	MPN
3cm	12	266	12	222	10	662	31	703	22	476	10	618	31	870	41	549	16	288
6cm	15	188	16	330	10	132	44	303	26	139	14	351	32	500	48	691	17	474
9cm	19	569	9	349	12	524	25	674	23	472	19	214	41	69	54	427	29	605
SR by compartment	26		20		18		60		33		23		47		57		29	
SRT	27						65						73					
Total by column MPN	1023		902		1318		1679		1087		1183		1439		1667		1368	

N/AMF presented in total, 3,950 $i g^{-1}$ sdw (Table 1). Root-zone showed a MPN = 1,679 $i g^{-1}$ sdw. CI showed 703 $i g^{-1}$ sdw at 3-cm depth (Table 1). Morphotype VIII exhibited 603 $i g^{-1}$ sdw at 9-cm depth for CI. Morphotype VIII dominated at 3-cm and 9-cm depth from CII. Morphotype IV (*Saccamoeba*: 329 $i g^{-1}$ sdw) at 3-cm from CI. Morphotypes II and III showed 191 and 226 $i g^{-1}$ sdw, respectively, from CIII at 3-cm depth (Fig. 2b; χ^2 , $P = 0.05$; Table S3).

AMF treatment showed 4,473 i g^{-1} sdw (Table 1). Although root-zone displayed 1,439 i g^{-1} sdw, and CII showed 1,667 i g^{-1} sdw, in total. CI showed 870 i g^{-1} sdw at 3-cm, in which morphotypes III, VI, X (*Tetramitus*) and VIII registered 224, 149, 118 and 114 i g^{-1} sdw at 3-cm depth Morphotype X was dominant at all depths (112 to 217 i g^{-1} sdw) from CII, and morphotype V (*Mayorella*) exhibited 191 i g^{-1} sdw at CIII (Fig. 2c; χ^2 , $P = 0.05$; Table S3).

Distribution And Species Richness

Control treatment registered 27 species (21 genera from 9 families; Table S4). Species richness along depths stayed constant, except for CI (Table 1). One species was exclusive in this treatment.

N/AMF treatment recorded 65 species at 30 genera from 19 families (Table S4). CI presented 44 species at 6-cm depth while CII 26 species were recorded at same depth. In contrast, CIII showed the fewest species richness (Table 1). Seventeen species were registered exclusively in this treatment.

For AMF treatment, 73 species were registered, which consisted in 34 genera from 18 families (Table S4). CI showed 41 species at 9-cm depth. CII registered 54 species at 9-cm depth (χ^2 $P=0.05$; Table 1). This treatment showed 20 species as exclusive.

Proportion And Diversity Trophic Groups

Regardless sampled depth and compartment, the most abundant trophic group was bacterivorous (B) amoebae (>60%) at all treatments (Fig. 3a,b,c). the fungivorous (F) trophic group only was found at 3-cm depth from AMF treatment, when compared to Control and N/AMF treatments in which the proportion of this trophic group was about 20% (Fig. 3b). In general, protozoa-eater amoebae (P) was registered in all treatments and compartments, excepting at CIII from AMF treatment at any sampled depth (Fig. 3c).

For control treatment, trophic diversity showed highest values for H' and D' indexes (1.2 and 0.9, respectively) at CI and CIII (Table S5), but the highest value of $H'=1.4$ was recorded from CIII at 9-cm depth (Fig. 3d, Table S6). In contrast, N/AMF treatment showed highest H' and D' indexes (1.3 and 0.75, respectively) at CI and CIII, which were similar to those indexes estimated for AMF treatment ($H'=1.4$ and $D'=0.6$) at CI (Table S5). Regarding sampled depth, N/AMF showed values of $H'=1.4$ at 6-cm depth, and $D'=0.7$ at 3-cm depth, both from CI. For AMF treatment the highest value of H' (1.2) was achieved at 3 and 9-cm depth from CI, whereas for 6 and 9-cm the H' value was similar (1.2) at CII (Fig. 3d; Table S6).

Beta diversity

Species compositional change (Whittaker descriptor) among compartments from N/AMF treatment was $\beta_w = 2$; whereas for treatments Control and AMF the β_w values among compartments, ranged between 1.2 and 1.4, respectively. In N/AMF treatment, β_w was of 1.2 among 3 and 6-cm depth from CIII; but for CI the β_w was of 1.3 at between 6 and 9-cm depth (Fig. 4). For AMF treatment, β_w value was of 1.3 between 3 and 6-cm depth, for CIII (Fig. 4a). Overall, the β_w values for the control treatment were as follows: CIII > CII > CI; for N/AMF treatment values were CIII > CI > CII; and for AMF treatment the β_w values were CIII > CI > CII (Fig. 4a; Table S7). Regardless sampled depth and microcosm compartments, β_w values for the treatment effect were as follows: N/AMF (2.0) > AMF (1.8) = Control (1.8) (data not shown).

Beta diversity with specific gradient (sampled depth)

Cody index (β_C) from Control treatment ranged from 7 to 5 among the three sampled depths from the three compartments (Fig. 4b). The β_C value of CI from treatment N/AMF was of 16, but at their adjacent compartments (CII and CIII) showed lower values (Fig. 4b). In AMF treatment, β_C values ranged from 8 to 11, and the highest value was recorded at CII among 6 and 9-cm depth (Fig. 4b; Table S7).

Control treatment showed a beta turnover index (β_T , Wilson & Schmidha) of 0.5 between 3 and 6-cm depths, at CIII. N/AMF recorded a $\beta_T=0.6$ in average among 6 and 9-cm depth at CI; whereas for AMF treatment the highest value of β_T (0.4) was achieved from 3 and 6-cm depth, at CIII (Fig. 4c).

Cody index β_{C_0} from Control treatment was of 0.5 in average, from 3 and 6-cm depth, at CIII. For treatment N/AMF the β_{C_0} value was 0.6 between 3 and 6-cm depth, at CIII; whereas for treatment AMF the β_{C_0} value was of 0.5 between 3 and 6-cm depth, at CIII (Fig. 4c; Table S7).

Bacterivorous Amoebae Beta Diversity

β_w value for bacterivorous amoebae from Control treatment, was 1.7, which was recorded at CIII, between 3 and 6-cm depths; whereas for N/AMF and AMF treatments the highest value of β_w was 1.4 and 1.3 from 3 and 6-cm and 6-9-cm depths, at CIII (Fig. 4d; Table S8).

Regarding specific depths gradient, bacterivorous beta diversity showed the following highest values: Control, $\beta_C = 7$ between 6 and 9-cm depth, at CI; N/AMF, $\beta_C = 16$ among 3 and 9-cm depths, at CI; and AMF, $\beta_C = 11$, between 6 and 9-cm depth, at CII (Fig. 4e; Table S8).

The β_T index value for Control, N/AMF and AMF treatments were around of 0.5 from the same 3 and 6-cm depths, at CIII (Fig. 4f). Moreover, β_{CO} values for N/AMF treatment was of 0.6, while for AMF and Control treatments, this value was around of 0.5, at CIII (3 and 6-cm depth) (Fig. 4f; Table S8).

AMF mycelium length and Pearson Correlation Coefficient with MPN and species richness of amoebae

From AMF microcosm, the longest AMF-mycelium length (19 cm) was achieved in CI at 9-cm depth; however, for CII showed the longest mycelium length (9.5 cm) was recorded at 6-cm depth, whereas for CIII, the longest mycelium length (6.5 cm) was registered at 6-cm depth. No AMF-mycelium was achieved from C (Non-planted) neither N/AMF microcosms (data not shown).

By considering data from AMF microcosm, the CI showed a negative correlation between the NMP of amoebae with the AMF-mycelium ($\rho = -0.9$; 81.7% determination), at 9-cm depth. In contrast, the correlation of mycelium length with amoebae species richness was positive 0.4 (14% determination). Species richness and length mycelium correlated $\rho = 0.9$ (80% determination) recorded in CIII (Fig. 5).

Similarity Analysis

Overall, Sørensen analysis revealed a core community composed mostly by bacterivorous; however, amoebae community composition changed when AMF was present (Fig. 6, B = red circles) when compared to C and N/AMF microcosms (Fig. 6).

Amoebae species composition was dissimilar at microcosms which plants were established (N/AMF and AMF); furthermore, this dissimilarity was observed among their own compartments (Control microcosms) (Fig. 6). Species composition from the microcosms N/AMF showed a cluster (CIII-6 and CIII-9, with 98% of similarity); moreover, this cluster shared 68% of their species with the amoebae community registered at CII-9. There was observed N/AMF, CI-3, CI-6 and CI-9 were dissimilarity of their species communities; furthermore, the remaining communities from CII, shared less than 81% of species (Fig. 6).

For AMF microcosm, the compartments CII-3, CII-6 and CII-9 configured a cluster with 87% of similarity, CII-6 and CII-9 shared 100% of their species this subgroup shared 63% their species with the communities from CI and CII at AMF y N/AMF respectively. Subgroup conformed of CIII-3 and CIII-6 (77%), but by adding the CIII-9 they shared 57% of species among these compartments (Fig. 6).

More specifically, bacterivorous communities from three depths shared species among all microcosms (AMF, N/AMF, and Control), if we considered those communities that shared at least 50% of their species, it is possible to found three different groups which are represented in the corresponding dendrogram (Fig S5a). Principal group was integrated by the communities from all treatments, but from this large group two 2 subgroups were detected. Other group was chained between communities founded from the AMF microcosms at the same compartment (CIII), regardless the sampling depth (Fig S5a). In contrast, communities registered for the Control microcosm had 62% similarity between CII and CIII, mainly at 3 and 6-cm of depth (Fig S12a).

Protozoa-eater amoebae community was formed four groups: the first one involves the control from three compartments with 100% similarity. The third and large group were integrated by AMF and N/AMF microcosms with 62% similarity (Fig S5b); moreover, AMF microcosms (CI) at 3 and 6-cm depth, showed 98% of similarity (Fig S5b).

Discussion

Amoebae vertical distribution of community was differentially affected by presence of either roots or AMF, and was dominated by bacterivorous amoebae.

Control presented the lowest species richness and abundance of amoebae individuals. However, species were heterogeneously distributed, being Amoebidae morphotype (VI) the most numerous. In this regard, bare soil is a heterogeneous environment in where microbial distribution depends on resources pockets which are heterogeneously distributed in soil matrix at microscale level. Moreover, moisture fluctuations and available food resources in edaphic microenvironments restrict both activity and reproductive capacity of protozoa [38]. These would explain the low similarity achieve for amoebae communities, which shared less than 30% of species among the compartments at unplanted microcosms (Control).

In contrast, N/AMF microcosms showed a significant increase of species richness, which was dominated by bacterivorous amoebae (morphotypes VIII and IV) around the root-soil (CI). *Vahlkampfia* and *Hartmannella* show preferences for certain bacterial species, and are capable of perceive, select, trace, or capture a great variety of preys [27]. In this case, roots without AMF increase amoeba species richness, thus, denoting an indirect “rhizosphere effect” on bacterivorous. Roots favor bacterial proliferation due to exudation of compounds that serve as carbon and energy sources [22]. This effect influence on the community of bacterial predators like nematodes and protozoa [28; 38], which may explain in part, the results obtained at N/AMF microcosms.

AMF microcosms showed the higher species richness and amoebae individuals at CII where only there was AMF-mycelium (hyphosphere) in comparison to CI in which both roots and AMF-mycelium (mycorrhizosphere) were presented. Either mycorrhizosphere or hyphosphere contributes on exudation of organic compounds rich on carbon and energy that enhance bacterial communities [9, 10, 12, 43, 44]. In consequence, community and trophic groups of amoebae also increase around these hotspots, as observed in our experimental conditions, in which species richness in CII (mycorrhizosphere) was 54, and three predominant trophic groups were identified as bacterivorous, protozoa-eaters, and algae-eaters.

Overall, vertical amoebae distribution showed greatest species richness at 6-cm depth at the root zone (CI) from N/AMF microcosms when compared to Control or AMF microcosms. The later reflects significant changes on amoebae communities at short distances of soil depths, especially in roots zones where there are available carbon sources for microbial activities [10, 39]. These organic compounds may be used by predators as signal molecules to find preys; thus, diverse interactions between plants and microbial food web increase around these zones [40, 41].

In general, the lowest amoebae richness and quantity of individuals was found at CIII from all microcosms. For microcosms N/AMF and AMF, root growth was restricted for CII and CIII, but, the effect of AMF-mycelium significantly increase the species and individuals bacterivorous (CII). Furthermore, hyphosphere influence extended beyond the roots to reach both CII and CIII, in which the average hyphal length was of 6.5 cm, at 9-cm depth.

Amoebae community around AMF-mycelium presented some species were exclusively found in this zone. Moreover, rare species were found in AMF microcosms (Table S4), and were different from those recorded at N/AMF and Control microcosms. In contrast, "common" species were very similar among the three microcosms (core community).

Amoebae are fourth element that plays part in soil-plant-mycorrhizal-microbiome interactions, and contributes on plant productivity by modelling the microbiome community, and by releasing nutrients [42]. This explains why the bacterivorous populations and protozoa-eater amoebae showed a significant increase in roots compartments (CI), at both N/AMF and AMF microcosms.

Furthermore, AMF sustain a different microbial species set as revealed by the amoebae community recorded in our research. Bacterivorous species number was greater in presence of AMF-mycelium, but fungivorous amoebae was undetected. It is probably that bacterial set cohabiting the AMF-mycelium may act as a "hidden scent" that helps on preventing activity of fungal predators [45], which may explain nonappearance of fungivorous amoebae at hyphosphere. Regardless trophic group, observed decrease on amoebae quantity at CI, had a negative correlation with presence of AMF in roots.

At trophic level, bacterivorous amoebae was dominant in all microcosms; however, their richness showed dissimilarities among microcosms. This indicates that all microcosms have similar functional activity of amoebae, but species identity changes among three microcosms.

Our results indicate that the greatest changes between communities (B_w) occurred at 3 and 6-cm depth, in CIII of the three microcosms. The communities change rate (B_C , B_{CO} and B_f) corroborates modifications in amoebae communities from N/AMF rhizosphere soil which showed higher change rate, at CIII. These differentiations among species composition of amoebae communities in three microcosms; are related to a complex environmental gradients induced by either rhizosphere or mycorrhizosphere, when compared to unplanted soil. Furthermore, both functional zones support larger species richness and amoebae number.

Conclusions

At short term experimentation, presence of *Rhizophagus intraradices* influenced species distribution into trophic groups of amoebae around the *Zea mays* root zone, but more significantly due to the presence of AMF-mycelium in root-free compartments. Structure of amoebae community around AMF-mycelium was different, and some species were exclusively found in mycelium zone. Moreover, rare species found in AMF microcosms were different to those recorded from N/AMF and Control microcosms.

Declarations

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Competing Interests

The authors declare no competing interests.

Author Contributions

Sandra Cortés-Pérez and Ronald Ferrera-Cerrato conceived and designed the experiments. Sandra Cortés-Pérez performed the laboratory experiments. Material preparation, data collection and analysis were performed by Sandra Cortés-Pérez. The first draft of the manuscript was written by Sandra Cortés-Pérez and all authors commented on previous versions of the manuscript. Alejandro Alarcón and Salvador Rodríguez-Zaragoza participated in critically reviewed the final manuscript, and all authors approved the final manuscript.

Data Availability

All data generated or analyzed during this study are included in this published article [and its supplementary information files as tables S1 and S3].

Ethics declarations

Not applicable.

Ethics Approval

Not applicable.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Rights and permissions

Not applicable.

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Figures

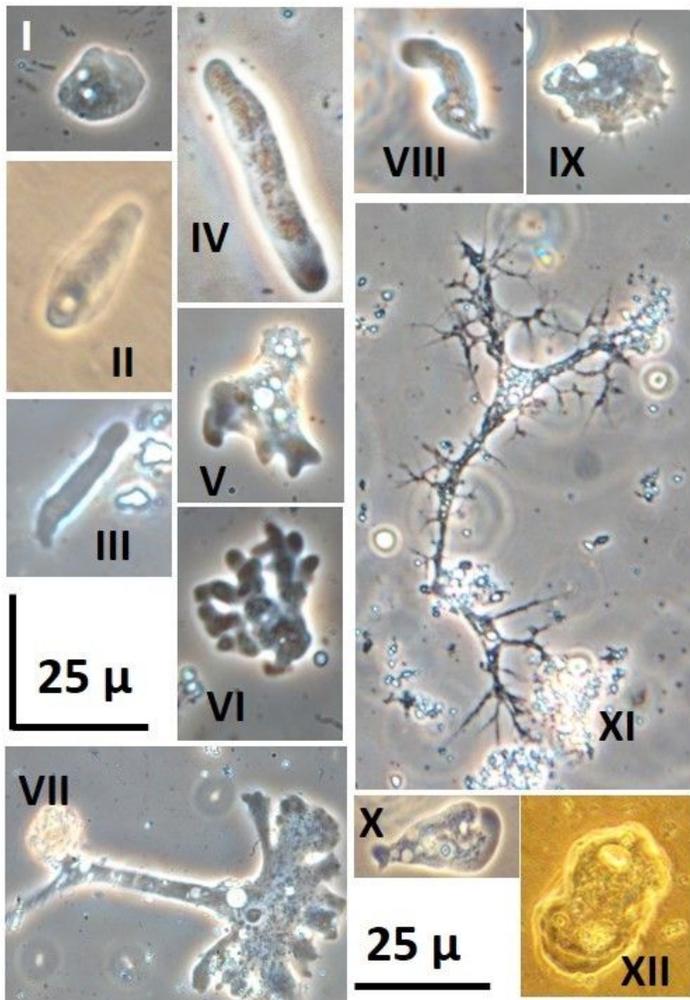


Figure 1

Amoebae categorization into 12 different morphotypes as follows: The first 4 types corresponded to limax form, no subpseudopodia. T I corresponded to fan-shaped-like amoebae, type species *Vannella platypodia*. T II to lanceolate morphotype-like, type species *Platyamoeba placida*. T III corresponded to worm-like amoebae, type species *Vermamoeba (Hartmannella) vermiformis*. T IV corresponded to monotactic morphotype, type species *Saccamoeba stagnicola*. T V corresponded to fan-shape amoebae with conical and/or mamilliform pseudopods, type species *Mayorella cultura*. T VI corresponded to polypoidal amoebae, type species *Polycaos* sp. T VII corresponded to web-like amoeba, type species *Leptomyxa* sp. T VIII corresponded to the limax amoebae with eruptive pseudopodia, type species *Vahlkampfia enterica*. T IX corresponded to *Acanthamoeba* sp-like amoebae. T X Amoebae showing a triangular form when fast-moving, with eruptive pseudopod, type species *Tetramitus* sp; T XI resembling a “root structure”, type species *Biomyxa* sp. and T XII amoebae includes the oval and wrinkled shape, type species *Thecamoeba terricola*

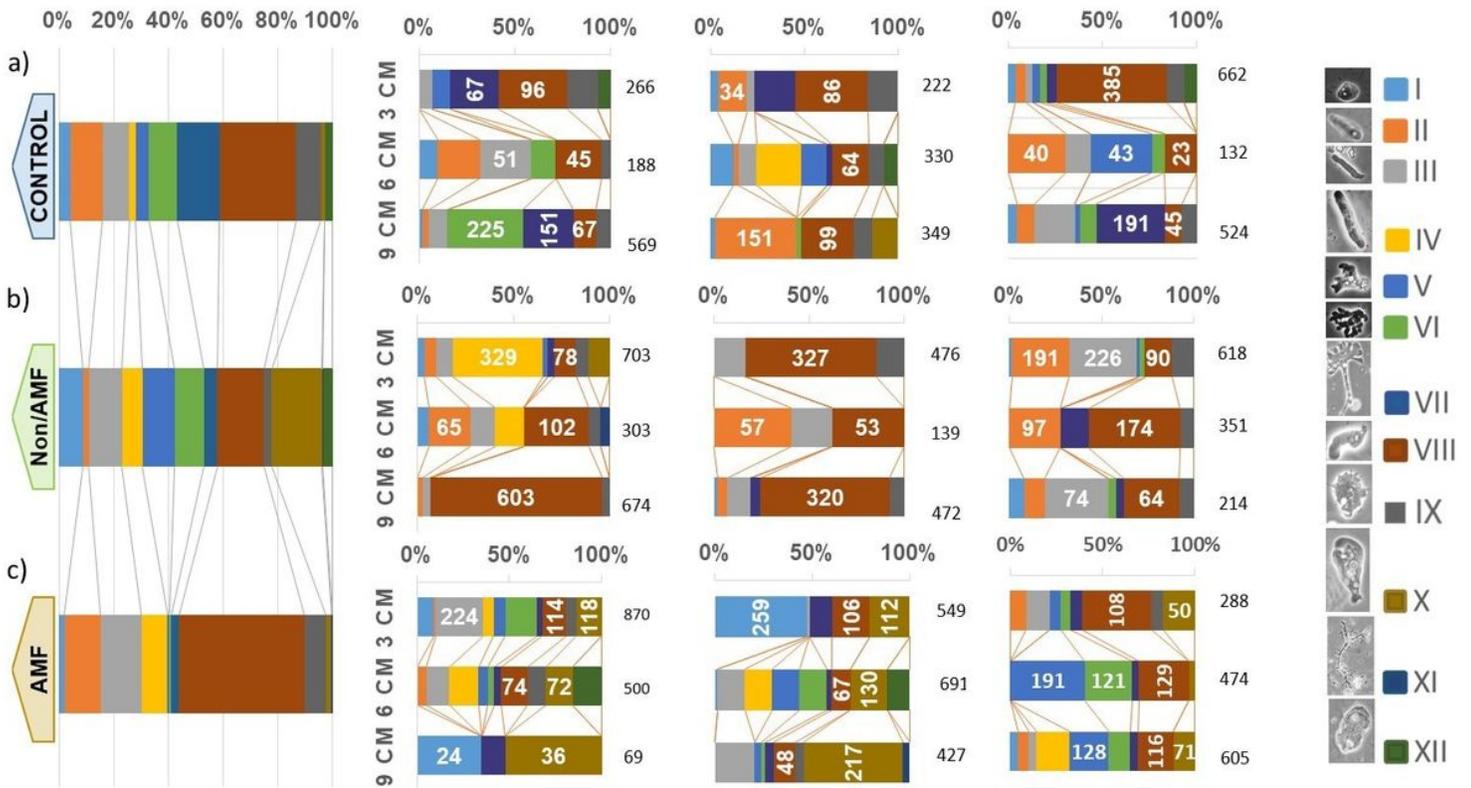


Figure 2

Relative abundance of 12 amoebae morphotypes from treatments and control group. Colors in banding pattern are the graphical representation of each morphotype relative abundance in control and treatments (3a control, 3b Non/AMF and 3c AMF), as well as by depths (3, 6 and 9-cm depth) and compartment of microcosms (CI, CII, and CIII). The 12 morphotypes are listed according to Fig. 2 as: T I; Vannella platypodia, fan-shaped-like amoebae. T II; Platyamoeba placida. T III; Vermamoeba. T IV; Saccamoeba stagnicola. None of the type I to type IV amoebae showed subpseudopodia (limax form); V; Mayorella cultura. T VI; polypoidal amoebae. T VII; Leptomyxa sp-like. T VIII; Vahlkampfia enterica-like. T IX; Acanthamoeba sp. T X; Tetramitus sp. T XI resembling a "root structure", Biomyxa-like and T XII; Thecamoeba terricola-like

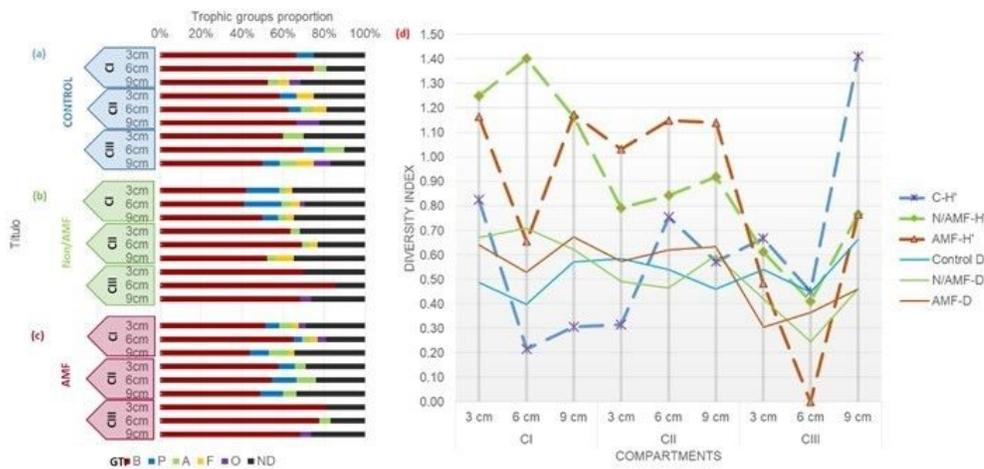


Figure 3

Relative importance of trophic groups (A). Abbreviations: N/AMF: treatment without mycorrhizal fungi. AMF: treatment with mycorrhizal fungi. Control (blue), N/AMF (Green) and AMF (Purple). The banding patterns are the graphical representation of the relative importance of each trophic group inside every depth (3, 6 and 9-cm) of each compartment (CI, CII, and CIII) of microcosms. The five trophic groups were present at 3-cm in the planted compartment (CI) of N/AMF and AMF treatments and in the 6cm depth of the N/AMF plants. Abbreviations: A, algivorous-amoebae; B, bacterivorous amoebae; F, fungivorous amoebae; O omnivorous amoebae; P, protozoa-eater amoebae and no feeding preferences determined or reported in the literature (ND). B) Trophic diversity index Shannon-Weaver (H') and Simpson (D) from control (Blue rows), N/AMF (green) and AMF (purple)

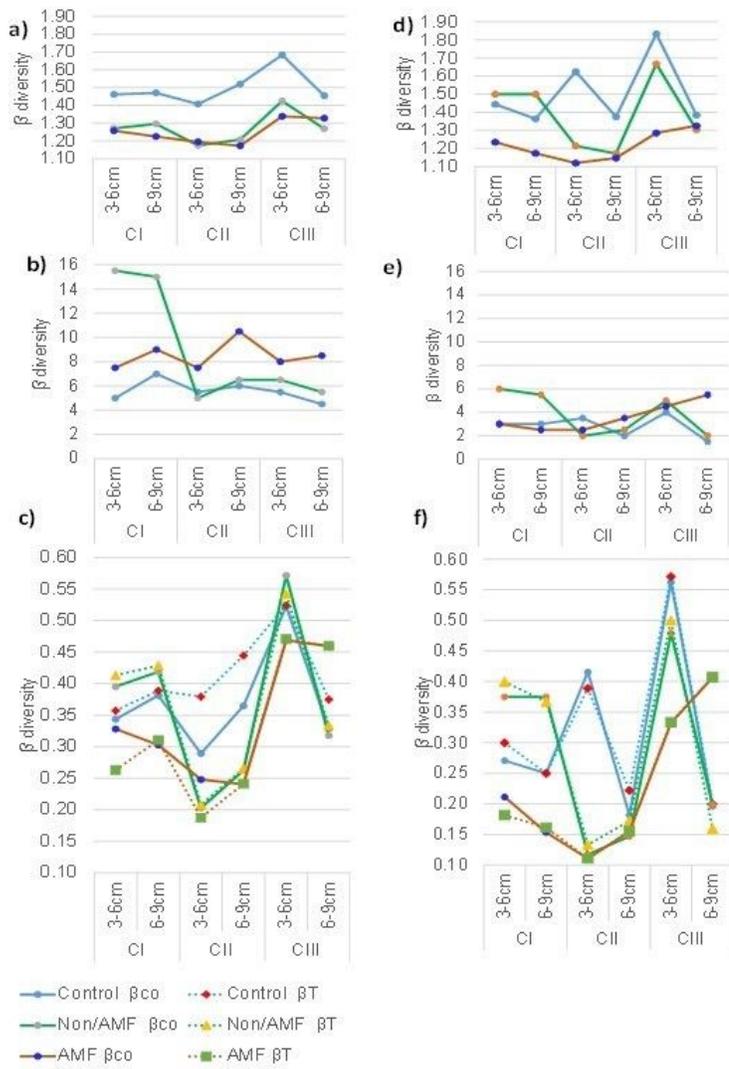
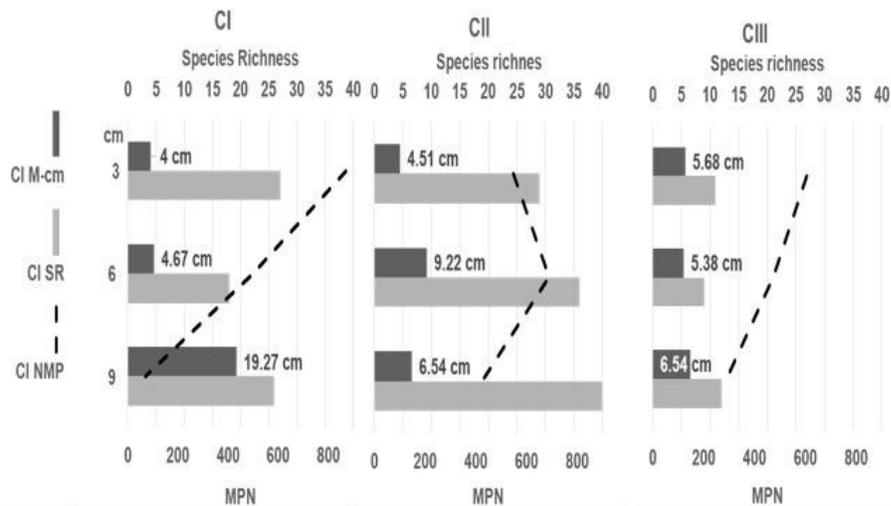


Figure 4

β -diversity values plotted against depths in cm to A-C show the values of communities from treatments calculated with presence-absence data. While the d-f graphs show β -diversity values of bacterivorous species from 3 - 6-cm depth and 6 - 9-cm depth from each compartment (CI, CII and CIII). Abbreviations: C = control group, blue line; N/AMF = treatment without mycorrhizal, green line; AMF; treatment with mycorrhizal, brown line. Graphics: A) Whittaker index or β_W (1992) ($0 \geq 2 =$ number of communities used for calculation). B) Cody index or β_C (1975). C) BCO = Cody index (1993) and Wilson & Shmida or β_T (1984). D) β_W calculation for bacterivorous guild ($0 \geq 2$). E) Cody index or β_C (1975) by bacterivorous guild. F) BCO and β_T (1984) bacterivorous guild



Mycelium Length	SR	Pearson	0.374	0.565	0.895
		Deter.	0.140	0.319	0.800
	MPN	Pearson	-0.904	0.602	0.643
		Deter.	0.817	0.363	0.413

Figure 5
 Mycelium length in cm (M-cm = black bar); Species richness (SR = black gray); MPN; Most probable number (broken line); AMF microcosms Pearson's correlation index between mycelium surface area, species richness and MPN. Abbreviations: CI, CII and CIII are the corresponding compartments of microcosms

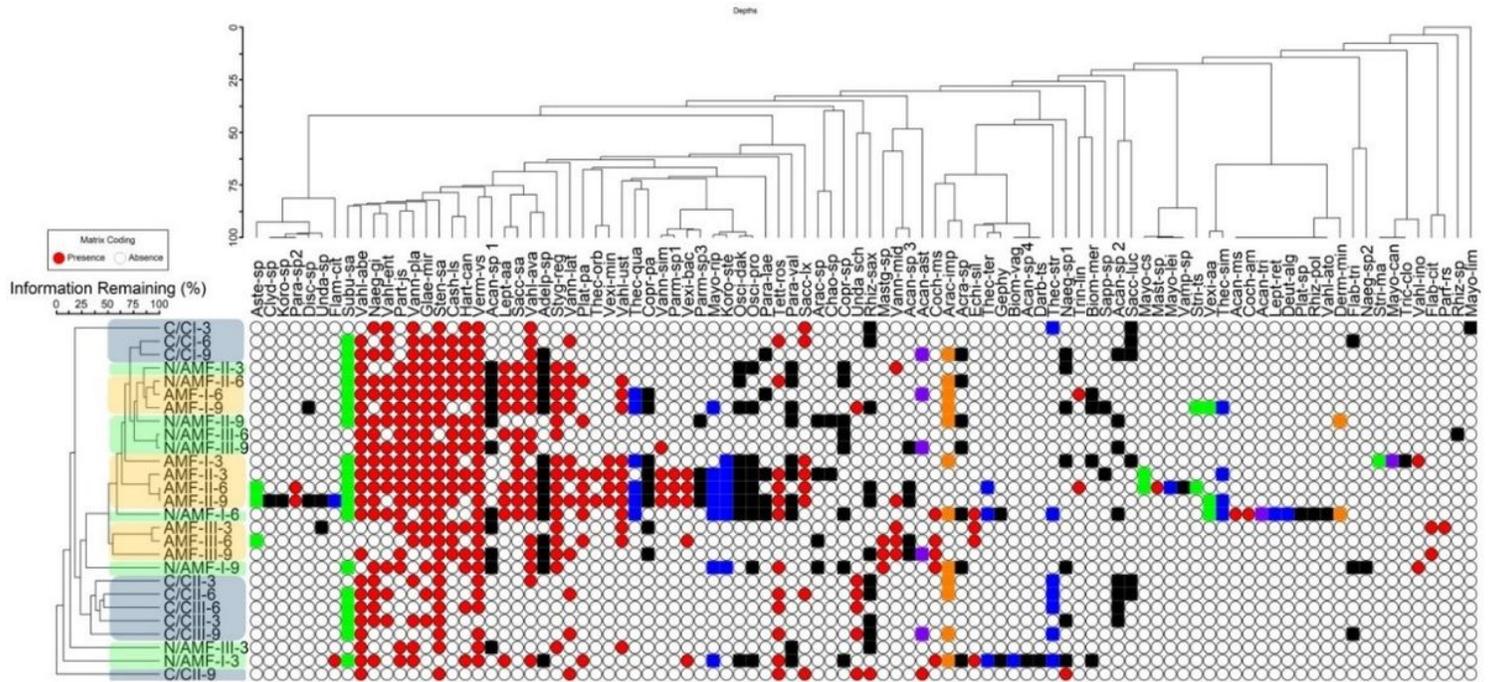


Figure 6
 Sørensen analysis of amoebae communities from treatments at different compartments and depths. Cluster analysis of amoebae communities showed the similarity between communities is due to the bacterivorous and algivorous groups in the first instance and by protozoa eater group in the second place. Captions at the dendrograms left side are compartments abbreviations for control and treatments as follows: C, unplanted-sandy-soil; N/AMF; planted-sandy-soil; AMF, mycorrhizal-plant. Latin number from the compartment of mycorrhizospheric-box and the Arabic number denoting

the depth of sample (3, 6 and 9). Color code is as follows: Green box = N/AMF treatment; Brown box = AMF treatment. Red circles: bacterivorous species. Blue circles: Protozoa-eater amoebae species. Green circles: algae-eaters species. Purple circles: omnivorous. Orange circles: fungivorous

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