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Effect of Earthworm, *Glyphidrilus* sp. on Soil Physico-Chemical Properties, Growth and Yield of Paddy - An *'In-Situ'* Pot Culture Experiment

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Abstract: A laboratory based observation was made during July - October, 2017 to study the role of *Glyphidrilus* sp. on the paddy (var. PAC 8744) growth and production through direct application of the earthworms in the potted paddy soils (*in-situ* earthworm technology). Control (without earthworms) and experimental pots (with earthworms), each with five replications were considered. Different paddy plant parameters viz. plant length, number of tillers plant^{-1}, total grains plant^{-1}, straw and root biomasses from both control and experimental pots were measured. *Glyphidrilus* sp. worked soils (experimental pots) had significantly higher ($p<0.01$) soil pH, Electrical Conductivity (EC), Cation Exchange Capacity (CEC), total nitrogen, available potassium and carbon contents and significantly lower ($p<0.01$) C:N ratio compared to the control pots. Experimental pot soil with *Glyphidrilus* sp. exhibited significantly the highest ($p<0.05$) plant length (122.4 cm), number of tillers plant^{-1}(24.4), total filled grains plant^{-1}(3985.68), straw biomass plant^{-1} (48.56 g) and root biomass plant^{-1} (15.89 g) compared to the control pot soils (plant length - 87.5cm, number of tillers plant^{-1} - 19, total filled grains plant^{-1} - 2088.5, straw biomass plant^{-1} - 31.76g and root biomass plant^{-1} - 6.21 g). Therefore, it can be concluded that earthworm *Glyphidrilus* sp. increases the macronutrient availability in soil and yield of paddy. Earthworm inoculation in the experimental pots led to two times increase in mean earthworm biomass following termination of experiment.

Key words: Cast-hill · Earthworm biomass · *Glyphidrilus* sp. · Paddy yield · Soil physico-chemical changes

INTRODUCTION

Earthworm's significance in plant growth has been perceived for over 100 years, through the book of Charles Darwin 'The Formation of Vegetable Mould Through the Action of Worms' [1]. Among all soil organisms, earthworms have been considered as a key functional group as they comprise the highest biomass among tropical soil macro-fauna involved in the process of decomposition, soil turnover, incorporating plant residues in the upper soil horizons, nutrient availability and thereby improving soil fertility and plant growth [2-4].

Earthworms enhance microbial population and activity and accelerate the process of rapid degradation of organic wastes and biodegradable materials and transform them into nutrient rich vermicasts which are useful in land fertility improvement and organic waste management [5, 6]. Earthworm casts are biologically active substance containing beneficial microbes and enzymes which greatly influence plant growth, soil aggregation and nutrient supply [7]. Worm casts hold more water than surroundings soils facilitating moisture absorption and accessibility to plants [8].

Rice is the main food crop of Tripura (22°51'–24°32'N, 90°10'–92°21'E), an agrarian state of North East India which covers almost 78-90% of cultivable area and produce 711.8 thousand tones of rice from an area of 254.3 thousand ha with an average productivity of 2.8 t ha^{-1} [9]. The dominant species of earthworm in paddy plantations in Tripura, is a semi-aquatic earthworm *Glyphidrilus* sp. which is found in the low wetland areas such as river bank mud, paddy fields etc. and produces a large accumulation of small tower shaped casts, termed as "cast hills"[10]. Population of these earthworm species inhabit the rhizosphere zone of paddy plants under sub-merged conditions and prefer to the paddy plant roots in soils as their suitable habitat. *Glyphidrilus* sp. is an endogeic earthworm species feeding on soils along

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with decomposed roots of paddy. Endogeic earthworms play an important role in soil turnover as they ingest and discharge large amount of inorganic soil particles in the form of casts [11]. Thus it is expected that *Glyphidrilus* sp. have a possible role in soil turnover as well as in paddy production. Several studies have been carried out on the effects of earthworms on plant growth and crop yield in terrestrial ecosystems [12 -14] but very few observations on their effect were made on aquatic habitats such as paddy fields [15, 16].

Based on the current scenario, the present investigation was carried out to study the role of the earthworm, *Glyphidrilus* sp. on the growth and yield of paddy through direct application of this earthworm in the wet paddy soils under pot culture ('*in-situ'* earthworm technology) and secondly to determine the effects of this earthworm species on the physico-chemical properties of paddy soil.

MATERIALS AND METHODS

Selection of Earthworm: Because of its dominance in paddy plantations, *Glyphidrilus* sp. (each individual of <1 g body weight, 5-10 cm length) was selected for this experiment. The earthworm and its habitat soils were collected from the soils of paddy plantations at Sekerkote (23°44'18"N, 91°15'48"E), West Tripura. Before introducing the worms in pot culture medium, they were acclimated in moisturized field soils in plastic bowl (30 L) under laboratory condition (temperature 27°C-30°C) for 15 days.

Pot Culture Preparation: Field soils (sandy clay) from paddy plantations, sundried for 3 weeks were brought to the laboratory, crushed with mortar and pestle and then sieved (1 mm) to remove cocoons and juveniles if any. Then 50 g dried cow dung (as organic additive) mixed with 30 kg of this soils was incorporated in each of the 10 plastic pots (5 control pots and 5 experimental pots). Water was added to the pots to maintain field moisture (70% - 90%) level.

Experimental Set: The study was conducted during July- October, 2017 on rice (var.PAC 8744). The experiment was set in a 5 m^2 area (having good aeration) over the roof of the Zoology laboratory building of Tripura University. The plot used for the experiment was covered with transparent polythene sheet supported by bamboo made framework in such a way to facilitate penetration of sunlight and also to protect from heavy rainfall. One pair of paddy plants (17 days old) were planted in each control (n = 5) and experimental (n = 5) plastic bowl (30L).

Introduction of Earthworms: Earthworms *Glyphidrilus* sp. (15-17 worm of 10 g biomass) were introduced in each of the 5 experimental pots (E_1- E_5) after one week of paddy plant transplantation. The control pots (C_1- C_5) were totally free from any earthworm. After the termination of the experiment, earthworms were collected from each experimental pot and their fresh biomasses (g) were measured.

Maintenance of Pot Cultures: Water was added every one day interval to keep up the moisten condition same as that of paddy fields in both control and experimental pots. The soils in the culture pots were covered with paddy straw as organic mulch to minimize the heat and loss of moisture from the culture pots. The temperature during the experimental period ranged from 27°C -30°C. The casts produced through earthworm activity were not removed from the experimental pots. During the entire experimental period, there was no further addition of soils or cow dung to any of the control and experimental pots.

Analysis of Plant Growth Parameters: Different paddy plant parameters such as plant length, number of tillers plant^{-1}, flowering stage plant^{-1}, number of panicles plant^{-1} etc. were taken every one month interval till the period of harvesting. A total number of grains (filled and unfilled), panicle length, straw and root biomass plant^{-1} were measured following harvesting.

Calculation: Yield (g plant $^{-1}$) = Number of productive tillers x number of filled grains x weight of 1000 grains x 1/1000 [16].

Soil and Cast Analysis: The experiment was terminated with the harvesting of paddy. The surface casts of *Glyphidrilus* sp. and surrounding soils from each experimental pot, as well as, from control pots were collected separately, air dried and ground with mortar pestle and sieved (1 mm). The soil samples were then subjected to analysis of different physico-chemical parameters. The pH was measured by digital pH meter (Eutech) using suspensions of the material in water in the ratio 1:2.5 (w/v). Total organic carbon was estimated by rapid titration method [17]. Total nitrogen and electrical conductivity (EC) and cation exchange capacity (CEC) were determined following standard methods [18]. Available phosphorus was determined in 0.5 M sodium bicarbonate extract adopting ascorbic acid colorimetric method [19] and available potassium in 1.0 M ammonium acetate extract by flame photometer [20].

Statistical Analysis: Variations in paddy plant height, number of tillers, flowering stage, paddy yield, panicle length, root and straw biomasses were tested by using two tailed paired sample t-test [21]. Significant differences ($p<0.01$) in the soil physico-chemical properties among initial and final stage of each control and experimental set, were determined by one way ANOVA followed by Tukey's post hoc test [22].

RESULTS AND DISCUSSION

In each experimental pot, good casting activities were noticed on soil surface in and around the paddy rhizosphere zone within 1-2 days following earthworm inoculation. In fact, surface casting is more significant than the subsurface casts in term of soil profile development and soil structure [23]. Earthworm surface casts can positively affect plant growth due to keeping up progressively nutrient materials like N, P, K, organic carbon than the soil [24]. The biological effect of earthworm cast is linked to microbial metabolites that impact plant metabolism, growth and development. Fulvic acids, humic acids and enzymes are present in abundance in the earthworm casts which enhance plant growth [25]. Humic acid provides binding sites for nutrients like phosphorus, potassium, iron, calcium etc. that are released on demands [26]. In live earthworm plant soil medium, the fresh enzymes, minerals and plant hormones are more readily available to plants [27].

Effects of *Glyphidrilus* sp. On Soil Physico-Chemical Properties: All the physico-chemical parameters viz. pH, EC(Electrical Conductivity), CEC(Cation Exchange Capacity), TN (Total Nitrogen), Av.K (Available Potassium) Av.P (Available Phosphorus) and OC (Organic Carbon) increased significantly ($p<0.05$) in both control and experimental soil at the end of the experiment when being compared with the initial stage (Table 1). Changes in the physico-chemical properties of soils in the control (without *Glyphidrilus* sp.) and the experimental sets (with *Glyphidrilus* sp.) with the termination of experiment are provided in Table (1). *Glyphidrilus* sp. worked soils with casts showed significant physico-chemical changes ($p<0.01$) in the experimental pots (E_1, E_2, E_3, E_4 and E_5) when being compared with the control pots (C1, C2, C3, C4 and C5) (Table 1). Earthworm introduction brought about significant increase ($p<0.01$) in soil pH (6.32), EC (340.2 μS/cm) and CEC (9.26 meq/100 g) in all the experimental pots than that of control pots (pH 5.62, EC 208 μS/cm, CEC 5.93 meq/100 g). Thus the percent increase in pH, EC and CEC were 12.45%, 63.55% and 56.15% respectively. Estimation of pH and EC gives important data to evaluating soil conditions for plant growth, nutrient cycling and biological activities. Increased soil pH in all the experiment pots was probably due to calcium carbonate secretion from the calciferous glands and also ammonia excretion from the body of earthworms. An increase in the EC of experimental pot soils was most probably because of increment in the level of dissolved salts which was due to gut-associated and cast-associated processes in earthworms [28-30]. Gradual increment in soil pH , EC and CEC in the experimental pots than control pots corroborates with the findings of Bisht *et al.* [3], Jouquet *et al.*[31], Chaudhuri *et al*. [28] and Choosai *et al*. [16]. There are reports [30] that increased levels of EC enhance the quality of crops.

Significant and maximum increment in soil organic carbon (from 0.82% to 1.15%), total nitrogen (from 0.31% to 0.71%) and available potassium (from 0.22 mg/100 g to 109.63 mg/100 g) was brought about by *Glyphidrilus* sp. in all of the experimental culture pots (Table 1). A total of 40.24%, 129.03% and 386.37% increase in carbon content, total nitrogen and available potassium of experimental soils were observed respectively. The carbon content of worm worked soil is likely to be higher than in the surrounding soils, to some extent because of the addition of intestinal mucus and mucus secreted from the body wall, but also because earthworms may select soil fractions enriched in organic compound [23]. Rice plants themselves do not fix atmospheric N_2. Heterotrophic N_2 fixer bacteria living close to or on the rice roots and also microbial biomass nitrogen can supply fixed N to rice plants amid growth period. Earthworm (*Glyphidrilus* sp.) activity can contribute to increase soil nitrogen through their urine and mucus [32]. Several authors reported a significant increase in nitrogen fixation in presence of earthworms and also recorded a greater nitrogen fixing activity in the earthworm burrows than the surrounding soils [32, 33]. Other than this, earthworms engulf a lot of plant organic matters that contain considerable amounts of nitrogen and great part of the nitrogen that they assimilate into their own tissues has returned back to the soil in their urine excretions (through nephridiopore).

Table 1: Showing the variations in the physico-chemical properties of soils in control and experiment pots

Soil parameters	Initial	Final Control	Final Experimental	F	P
pH	5.39 ± 0.005^a	5.62 ± 0.01^b	6.32 ± 0.04^c	56.2	< 0.01
EC (μS/cm)	179.8 ± 1.44^a	208 ± 2.21^b	340.2 ± 1.78^c	-15.42	< 0.01
CEC meq/100 g	4.96 ± 0.2^a	5.93 ± 0.12^b	9.26 ± 0.41^c	65.62	< 0.01
TN (%)	0.14 ± 0.03^a	0.31 ± 0.01^b	0.71 ± 0.03^c	130.6	< 0.01
AK (mg/100g)	16.59 ± 1.16^a	22.54 ± 0.46^b	109.63 ± 7.8^c	135.8	< 0.01
AP (mg/100g)	0.99 ± 0.03^a	1.15 ± 0.01^b	1.22 ± 0.04^b	10.97	>0.01
Org. C (%)	0.56 ± 0.03^a	0.82 ± 0.02^b	1.15 ± 0.02^c	87.6	< 0.01
C/N Ratio	4.13 ± 0.4^a	2.69 ± 0.11^b	1.64 ± 0.05^c	17.67	< 0.01

Different letters (a, b, c) denotes significantly different at 1% level of significance

The latter contains mucoproteins secreted by gland cells present in the epidermis and urea, ammonia and perhaps allantoin. These excreatory substances contribute a significant amount of readily assimilable nitrogen to soils [32]. In fact, experimental pots having a noteworthy level of nitrogen and potassium than control pots were mainly due to gut transit in earthworms which increases mineralization converting nitrogen and potassium into forms that are effectively accessible to plants [34]. Elevated level of potassium in *Glyphidrilus* sp. worked soil may also be due to selective feeding of earthworms on foliar or other food materials enriched with this cation. A significant increase in concentration of available potassium in the casts of *Metaphire houlleti*, *Kanchuria* Sp.1 and *E. gigas* than their surrounding soils has earlier been reported [28]. Apart from this, rice straw can be a good organic reservoir of potassium, nitrogen and phosphorus. According to one recent report [35], rice straw aggregate approximately 80%- 85% of the K, 40% of the N and 30%-35% of the P during crop maturation. Thus, presence of rice straw can be a conceivable explanation behind remarkable increment in potassium and nitrogen level in experimental pots compared with control pots, as in both control and experiment pots rice straw was used as a natural mulch to minimize the heat and moisture loss in soils. Due to presence of earthworm activity in the experimental pots, rice straw decomposed rapidly than the control pots where earthworms are absent. Several other workers also reported about the significant increase in total soil nitrogen and potassium after incorporation of earthworms and vermicasts as soil amendment [3, 7, 16, 11, 5]. A remarkable increase in the available potassium level in presence of earthworms in sludge soils and casts in rubber plantations were reported by Bhattacharjee [36] and Chaudhuri *et al.* [28] respectively.

Although there was an increase in the amount of av. P (from 1.15 mg/100g to 1.22 mg/100g) in the worm worked soils but the change was not significant ($p>0.05$). Only 0.06% increase was observed in experimental pots (Table 1). The absorption of av. P by rice plants in the experimental pots is the most probable reason for this. In a greenhouse experiment, Mackay *et al*. [37] reported the influence of earthworms on the uptake of phosphorus by perennial ryegrass over seven consecutive harvests of the gram. An increment in the amount of soil nutrient elements like nitrogen (N), phosphorus (P) and potassium (K), which are required in relatively large amounts by the plant, is the major factor behind betterment of paddy plant growth.

C: N ratio is a measure of degradation and net mineralization of soil nutrients. There was a gradual decline in C: N ratio in the experimental pots (1.64) in contrast to the control (2.69). Earthworms probably decrease the C:N ratio in soil because they increase combustion of carbon by enhancing total soil respiration [32].

Nitrogen plays a remarkable role in growth, yield, photosynthesis, cell division and differentiation, growth and somatic embryogenesis, chlorophyll content, photosynthetic rate following fruiting, maturation and is a significant element of proteins required for the metabolic processes that occurs during plant growth [38].

Potassium is often referred to as the "quality element" for crop production [39-40]. An appropriate amount of K is required by plants to facilitate adequate root growth, proper seed development, higher yield and quality of crop and assumes a role in numerous physiological procedures essential for plant development, including the maintenance for plant water balance and protein amalgamation. Potassium mediated resistance against biotic stresses such as those caused by microbes and insect pests are also well explained [41]. In case of rice plant, potassium requirements is quite high even greater than that of nitrogen [42].

Table 2: Showing the variations on different paddy plant parameters in control and experiment Pots (Mean ± S.E.) at the termination experiment

Parameters	Control	Experimental	T-value	P-value
Total number of filled grains plant^{-1}	2088.5 ± 118.32	3985.68 ± 144.9	2.71	P<0.05
Total number of unfilled grains plant^{-1}	854.2 ± 36.45	743.8 ± 34.62	-2.92	P<0.05
Total weight (g) of filled grains	46.41 ± 2.22	101.51 ± 1.88	14.24	P<0.01
Appearance of flower (Days)	28.4 ± 0.24	26.4 ± 0.24	4.77	P<0.01
Total tillers plant^{-1}	19 ± 1.18	24.4 ± 0.5	3.87	P<0.05
Length of panicle (cm) plant^{-1}	20.31 ± 0.94	26.93 ± 0.55	-7.013	P<0.01
Yield (g) plant $^{-1}$	60.54 ± 3.85	190.51 ± 14.42	10.91	P<0.05

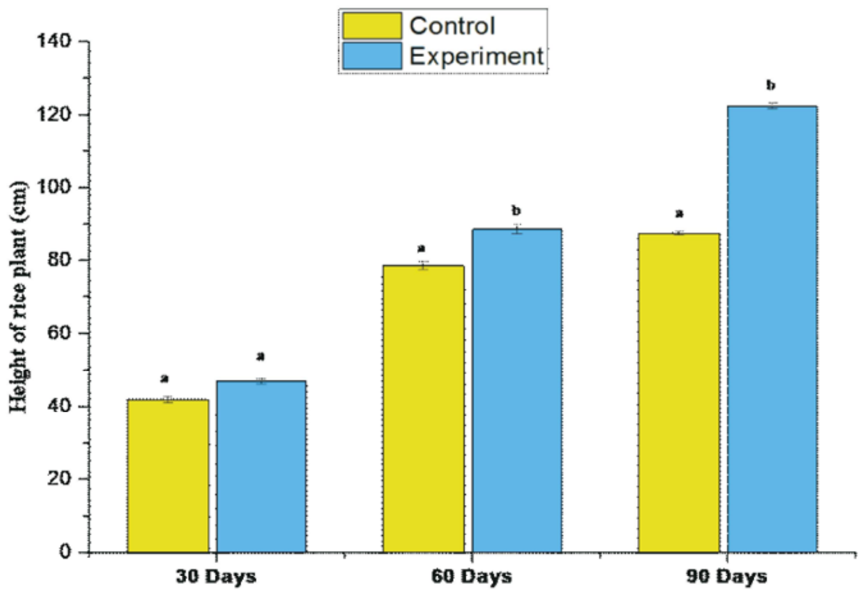

Fig. 1: Increase in height of rice plant (cm) in monthly intervals [different letters (a, b) denotes significant difference at 1% level]

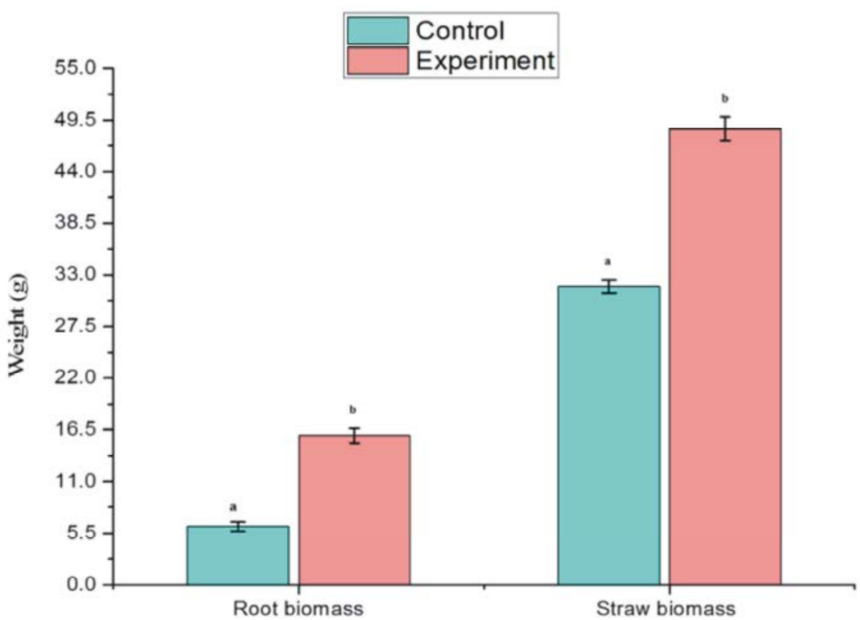

Fig. 2: Biomass (g) of root and straw of paddy in control and experimental pots [different letters (a , b) denote significant difference at 1% level]

The phase of most elevated phosphorus take-up is the young panicle developmental stage pursued by the tillering stage, however, much P is likewise assimilated at the maturity stage [43] also plays an important role in increasing water-use efficiency. It is a critical factor for plant productivity in dry conditions improves leaf extension, axillary bud development and shoots canopy, improved photosynthetic surface region and carbohydrate utilization [44].

The nutrient absorption amount varies with rice growth stage; each macro nutrient is required at different phases of rice growth [43].

Effect of *Glyphidrilus* sp. And Their Castings on the Paddy Plant Growth and its Yield: *Glyphidrilus* sp. following inoculation in the soils of experimental pots had significantly influenced the overall growth parameters of the paddy plants *viz.* plant length (Figure1), number of tillers plant^{-1}, flowering stage plant^{-1}, length of panicle plant^{-1} and total weight of filled grains plant^{-1} (Table2) etc. Paddy plants in experimental pots became mature earlier than that of paddy plants in control pots (Table 2). After harvesting, there was significant ($p<0.01$) increase in the paddy yield in all the treatment pots with earthworm compared to the control pots without earthworms (Table 2). Biomasses of straw (48.56 g) and roots (15.89 g) also increased significantly ($p<0.01$) in all the experimental pots compared to the biomasses of straw (31.76 g) and roots (6.21g) in control pots at the end of the experiment (Figure 2). Earthworms burrowing movement in soils favour root development, plant improvement and crop yield [45]. Bisht *et al*. [3] observed 65% increase in maize yield, 58.1% increase in wheat yield and 61.7 % increase in the yield of barley in earthworm (*Octolasion tyrtaeum*) treated soils under laboratory conditions. Earthworm burrows acts as an ideal channel for the growth of plant roots [32]. In a series of pot and box experiment, Atlavinyte and her co-workers [46, 47] demonstrated clearly that there is a strong correlation between the number of earthworms (*A. caliginosa*) and the growth of barley. Atlavinyte [48] further recorded that addition of straw with earthworms increased barley yields more than addition of earthworms only. In a green house experiments in Australia, *A. trapezoids* and *A. rosea* increased the shoot and root growth of wheat plant and also increased the concentration of many nutrients in the wheat shoots [49]. Production of roots and their penetration in deeper soil layers was significantly greater in earthworm inoculation plots [50]. In his review Scheu [13] claimed that in majority of the observations (79%) shoot biomass was expanded in the presence of earthworms. Dramatic increase in straw production in 75% plants and 25% increment in the crop yield in presence of earthworms have recently been reported [51] . The growth of rice plants is positively affected in presence of vermicast which is a natural source of plant supplements and contains a higher level of plant accessible nutrients necessary for plant growth [52]. Owa *et al.* [15] reported faster rice developments and greater productivity when earthworm casts were associated with rice plants. The presence of earthworms and their casts significantly increase plant growth is also reported in recent studies [3, 53, 16, 54].

Recovery of earthworms following termination of experiment: After initial inoculation of 10g biomass (young and adult) of *Glyphidrilus* worms in each experimental pots, a mean biomass of 19.12 ± 0.51 worms (density 25 to 30 individuals) and a large number of juveniles (intimately associated with bushy roots of paddy plant) were recovered from worm worked soils. Increase in density and biomass of earthworm with large number of juveniles in the culture pots at the end of the experiment was due to their growth and reproduction in the paddy soil culture medium.

According to Scheu [13] earthworms modify nutrient accessibility to plants and change the entire rhizosphere condition. The mechanism by which earthworms influence plant growth comprise of direct impacts, for example, root feeding. He further emphasized that plant development is modified predominantly by changing the soil structure, mineralization process, hormone like impacts, dispersal of plant growth promoting microbes and also dispersal of microorganism antagonistic to root pathogens. Earthworms, in fact, govern the distribution of organic matter in soil and therefore seem to affect root foraging.

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