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Earthworms and root-knot nematodes: effect on soil biological activity and tomato growth

Minhocas e nematoides das galhas: efeitos na atividade biológica do solo e crescimento do tomate

Wilian Carlo Demetrio^{1*}; Jair Alves Dionísio²; Arlei Maceda³

Abstract

Earthworms are a representative soil invertebrate, and their living habits are known to influence a large diversity of organisms. The objective of this study was to evaluate the ability of *Amyntas* spp. to change the biological attributes of soil, and its potential to reduce infection by root-knot nematodes on tomato crop. The study was conducted in the greenhouse of the Diagnostic Center Marcos Enrietti, Federal University of Paraná, Brazil. The treatments earthworms at the following densities: control (absence of earthworms), two, four, six, and eight, which were inoculated into different pots, with five replicates per group. In each pot, a single tomato plant (*Solanum lycopersicum*) was used, and a suspension of *Meloidogyne javanica* containing 3000 eggs and/or juveniles was added 14 days after seeding. During the experiment, edaphic respiration was evaluated at 96-h intervals. After 91 days, soil microbial biomass carbon (MBC), microbial soil respiration (MSR), the metabolic quotient (qCO_2), dry mass of roots (DMR), dry mass of plants (DMP), and the number of root galls were determined per plant. We observed that inoculation with higher earthworm densities increased the MBC. Furthermore, the lowest earthworm density (two animals) resulted in a MBC that was 75% higher than that of the control treatment (earthworms absent). There was a positive correlation between MBC and DMP, and a negative correlation between MBC and qCO_2 . The DMR was not influenced by inoculation with earthworms. A linear increase in DMP was observed with earthworms; however, gall formations on the tomato root were not suppressed.

Key words: *Amyntas* spp. Edaphic respiration. *Meloidogyne javanica*. Microbial biomass.

Resumo

As minhocas são um dos mais representativos invertebrados do solo e sabe-se que seus hábitos de vida influenciam uma grande diversidade de outros organismos. O objetivo deste estudo foi avaliar a capacidade de *Amyntas* spp. em alterar alguns atributos biológicos do solo e seu potencial em reduzir a infecção de nematoides formadores de galhas na cultura do tomate. O estudo foi conduzido em casa de vegetação no Centro Diagnóstico Marcos Enrietti, Universidade Federal do Paraná, Brasil. Os tratamentos foram diferentes densidades de minhocas: Controle (ausência de minhocas), dois, quatro, seis e oito indivíduos inoculados por vaso, com cinco repetições. Em cada vaso foi utilizada uma única plântula de tomate (*Solanum lycopersicum*), onde, aos 14 dias após a semeadura foi adicionada uma suspensão contendo 3000 ovos e/ou juvenis de *Meloidogyne javanica*. Durante o experimento,

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a respiração edáfica foi avaliada em intervalos de 96 horas. Após 91 dias, o carbono da biomassa microbiana (MBC), respiração microbiana (MSR), quociente metabólico ($q\text{CO}_2$), massa seca das raízes (DMR), massa seca da planta (DMP) e o número de galhas por planta foram determinados. Como resultados, observou-se que a inoculação de altas densidades de minhocas aumentou o MBC. Além disso, baixas densidades de minhocas (dois indivíduos) mostraram valores de MBC 75% maiores, comparados ao tratamento controle (ausência de minhocas). Houve uma correlação positiva entre MBC e DMP, negativa entre MBC e $q\text{CO}_2$. A DMR não foi influenciada pela inoculação de minhocas. Um aumento linear da DMP foi observado com o aumento da densidade de minhocas, sem ocorrer supressão da formação de galhas nas raízes.

Palavras-chave: *Amyntas* spp. Biomassa microbiana. *Meloidogyne javanica*. Respiração edáfica.

Introduction

Earthworms are edaphic animals, known as “ecosystem engineers”, which are a group of animals able to alter the environment in which they live, can represent up to 80% of the biomass of soil organisms (LAVELLE et al., 1997).

The feeding behaviour of earthworms is diverse, ranging from detritivores to geophagous organisms (EDWARDS; BOHLEN, 1996). The former are responsible for the decomposition of organic waste, while the latter are responsible for the spatial displacement of organic and mineral particles in the soil profile, thus, changing the availability of food to other soil animals, especially the microbial community (BROWN et al., 2000).

The action of earthworms in soil is known to change microbial biomass and the activity of microorganisms (AIRA et al., 2007; BROWN et al., 2006 as well as soil respiration (HÖGBERG; READ, 2006). In addition, these animals can promote changes in the chemical (pH and nutrient availability) and physical (water retention capacity, infiltration, aeration, and soil structure) properties. Earthworms are saprophytic and feed on dead organic matter, and do not usually pose any detrimental effects to plant roots (BROWN et al., 1995). Additionally, they are capable of interacting with the edaphic microfauna, such as nematodes, protozoa, and rotifers, which can be ingested together with the soil because of the feeding habits of the oligochaetes (EDWARDS; BOHLEN, 1996).

Nematodes represent a large portion of the

soil microfauna. The *Meloidogyne* genus is characterised as a phytonematode because of its feeding behaviour, and is known to parasite plants and induce gall formation (MOENS et al., 2009). In Brazil, nematodes that form galls are responsible for losses in grain crops, oilseeds, fruit, and ornamental plants (MOREIRA; FERREIRA, 2015).

Many management practices have been employed to control nematodes, such as the use of non-host plants of these organisms during crop rotation and the solarisation of soil. However, the effectiveness of such management practices is limited, mainly due to the high resilience of these animals (MÔNACO et al., 2008; RADWAN et al., 2012). Moreover, the use of chemical nematicides, in addition to being expensive, could result in soil and environment contamination and cannot be used in organic production systems (NEVES et al., 2009).

The tomato (*Solanum lycopersicum*) is one of the most produced vegetables in Brazil and in the world. The Paraná, State South Brazil, is the 4th largest domestic producer of tomato (IBGE, 2015). This culture is sensitive to nematode attack, and losses due to the partial loss of root function range from 14 to 44% (BELAN et al., 2011; CHARCHAR; ARAGÃO, 2005).

Little is known about the potential suppression of nematodes by earthworms. Lafont et al. (2007) studied the interaction between *Pontoscolex corethrurus* and nematodes of the *Radopholus* genus. They observed that the presence of earthworms did not reduce the number of nematodes on the banana

root system, but enhanced plant growth. Moreover, some studies have reported significant results using *Eisenia andrei*, including the reduction in the nematode population in a vermicomposting system (DOMÍNGUEZ et al., 2003). A 51% reduction in the nematode population in soil was observed following the addition of *Lumbricus rubellus* (ILIEVA-MAKULEC; MAKULEC, 2002). In Brazil, a study by Dionísio et al. (2014) showed a reduction of more than 50% in the total number of nematode galls (*Meloidogyne paranaensis*) on tomato roots, using *Amyntas* spp.

The objective of the present study was to evaluate the ability of *Amyntas* spp. to suppress the parasite population of nematodes, and to examine the extent of the impact of these earthworms on soil biological activity and tomato growth.

Material and Methods

The study was conducted in a protected environment at the Diagnostic Center Marcos Enrietti, located in the Sector of Agricultural Sciences - Federal University of Paraná (UFPR). A Cambisol clay loam texture soil was used as the substrate (EMBRAPA, 2013). Initially, the soil was sieved (4-mm mesh) and sterilised in a steam oven, at approximately 100 °C for 3 h, then packed in plastic polyethylene pots. The substrate was characterised as follows: pH of 5.70 CaCl_2 0.01 M; exchangeable cations ($\text{cmol}_c \text{ dm}^{-3}$) Ca 8.40, Mg 4.80, and K 1.07; P (mg dm^{-3}) 43.9; C (g dm^{-3}) 33.3; and V (%) 76; and physical (g kg^{-1}); sand 420, silt 305, clay 275; and water retention capacity 38%. The experimental setup was divided in two stages as described below.

Stage 1 - Production of *M. javanica* inoculum

Seedlings of tomato (*S. lycopersicum*, variety Santa Cruz Kada Paulista) were produced in 250 mL plastic pots, containing the commercial substrate pine bark Mecplant®, biostabilised, homogenised with <2-mm particles without fertiliser, and

sterilised. At 14 days after emergence (DAE), the plants were transplanted to plastic pots (PP) with a volume of 4 L, containing 3 kg of soil. One week later, plants were inoculated with an aqueous suspension containing eggs and/or juveniles (no count) of the nematode *M. javanica*. The plants were left for approximately 180 days for inoculum production.

Stage 2 - Experiment

The experiment was conducted in a completely randomised design using earthworm density as the main factor, with five treatments T1 = 0, T2 = 2, T3 = 4, T4 = 6, and T5 = 8 earthworms (*Amyntas* spp.) per pot and five replicates. The experimental units (EU) were each represented by a 4-L PP, containing 3 kg of soil and a single inoculated adult worm.

The adult animals were collected manually in the UFPR Sector of Agricultural Sciences, and were selected by the presence of clitellum and identified to genus level (SIMS; GERARD, 1999). The earthworms were rinsed with deionised water, dried with paper towels, and fresh biomass (FBE) was recorded. FBE (g) used in the treatments T2, T3, T4, and T5 was 2.616 ± 0.208 ; 4.959 ± 0.3688 ; 7.687 ± 0.9776 , and 9.512 ± 0.3984 , respectively.

Each EU was kept for 10 days covered with nonwoven fabric, to ensure the acclimatisation of earthworms, preventing them from escaping, and allowing deaths to be verified and animals to be replaced, if necessary. After acclimatisation, the tomato variety Santa Cruz Kada Paulista was sowed in PP, and then thinned at 14 DAE, leaving one plant per pot.

The nematodes were inoculated onto plants 3 days after thinning. Each EU received an aqueous suspension containing 875 eggs and/or juveniles of *M. javanica*, which were obtained according to the methodology described by Bonetti and Ferraz (1981). *M. javanica* were mashed in a blender with NaClO solution (0.5%) and sifted (0.075 and 0.025

mm). Nematodes were counted under an optical microscope (25× amplification). Each EU was inoculated with 3.5 mL of the solution containing *M. javanica*, which were distributed in three 1-cm holes on the stem of plants.

During the experiment, the plants received daily irrigation, pruning, and nitrogen fertilisation in line with CQFS-RS/SC recommendations (SBCS, 2004). At the end of the experimental period (91 days), the plant stem was cut to 1 cm above the soil surface and dried at 65°C to determine weight.

The remaining earthworms in each EU were collected manually, washed with deionised water, and used to quantify fresh biomass. From each EU, 400 g of soil was collected and stored at 2-4 °C for analysis of microbial biomass carbon (MBC) and microbial soil respiration (MSR).

The roots were separated from the soil by rinsing with water and kept in a biochemical oxygen demand (BOD) incubator at $2 \pm 0.5^\circ\text{C}$ to preserve plant tissues until galls were counted using a stereomicroscope (4× increase). Finally, the roots were dried at 65°C, and the mass was determined.

Edaphic respiration (ER) was determined using the methodology described by Grisi (1978), with adaptations. This methodology is based on the CO_2 emitted per area; for this, a respirometer (300-mL plastic cup) was used with another plastic container (PC) inside (50 mL), fixed 3 cm above the ground, containing NaOH (0.5 mol L^{-1}). The plastic containers were replaced at every reading, at 96-h intervals, and the temperature inside the EU was determined at a depth of 1 cm.

The ER was estimated by back titration ($\text{HCl } 0.5 \text{ mol L}^{-1}$) of NaOH excess along with BaCl_2 (50%), and phenolphthalein (0.1%). The C- CO_2 emitted per unit surface area was calculated according to the method described by Anderson (1982).

The MBC was determined by the *substrate-induced respiration* (SIR) method, as described by Anderson and Domsch (1978). From each sample,

50 g of dry soil was weighed and transferred to 1-L plastic containers (PCo), after which an aqueous solution (60 mg glucose) was added to increase the humidity to 40% of the water retention capacity of the soil. The PCo was tightly closed and preincubated for 2 h in an incubator at 22°C. After each one received a PC containing 10 mL of NaOH (0.5 mol L^{-1}), they were incubated under the same conditions for at least 4 h. At the end of the incubation period, the samples were titrated as described for the ER, and the MBC was estimated according to the method described by Höper (2006).

The MSR was determined as described by Alef (1995), with some modifications, in a static system using a PCo containing a 50-g sample of soil (dried) moistened to 40% field capacity. A test tube containing 10 mL of deionised water was placed into the PCo to maintain indoor humidity and prevent the sample drying, together with 50-mL PC containing 10 mL of NaOH 0.5 mol L^{-1} . The samples were incubated at 25°C for 168 h, and the excess NaOH was titrated as described for ER and MRS, and estimated according to the method described by Stotzky (1965).

The metabolic ratio ($q\text{CO}_2$) was determined using the estimated values for MBC and MRS, as described by Anderson and Domsch (1993) to determine the ratio of MRS/MBC.

The data obtained for the analysed variables were subjected to an extreme value test (Grubbs) to detect outliers. Subsequently, normality was assessed using Shapiro-Wilk test and homogeneity of variance was determined using Bartlett's test. Pearson correlation analysis and regression ($p < 0.05$) were applied to all variables. Model selection was based on the significance of the regression coefficients and the higher value of the determination coefficient.

Results and Discussion

ER showed a linear response in 54.5% of the readings (Table 1), demonstrating a positive

correlation with increasing earthworm density. This increase can be attributed to several factors, including soil burrowing by the animals, improvements in gas exchange in the soil-atmosphere system, which increase the O₂ flow, and facilitation of respiration by the roots (LI et al., 2002). Moreover, the presence of these animals

also stimulates the activity of soil microorganisms (SIMEK; PIZL, 2010) which may contribute more than 70% of the total ER CO₂ (ZHAO et al., 2013). The main effects of this stimulus probably occurred at depth (SIMEK; PIZL, 2010), where low activity has been observed compared with the surface layer (SANTOS et al., 2004).

Table 1. Regression analysis of soil respiration and different numbers of earthworms (*Amyntas* spp.) in the presence of nematodes (*Meloidogyne javanica*) on tomato crop (var. Santa Cruz Kada Paulista).

| Date | Read n° | Equation | r ² | CV % |
|------------|---------|-------------------|----------------|-------|
| 12/09/2014 | 1 | y = 20.20 + 0.60x | 0.90** | 10.22 |
| 15/09/2014 | 2 | y = 19.48 + 0.70x | 0.98** | 9.05 |
| 19/09/2014 | 3 | ns | ns | 13.53 |
| 24/09/2014 | 4 | ns | ns | 12.07 |
| 28/09/2014 | 5 | y = 19.44 + 1.23x | 0.89* | 8.00 |
| 02/10/2014 | 6 | y = 23.53 + 0.42x | 0.95* | 10.16 |
| 06/10/2014 | 7 | ns | ns | 13.70 |
| 10/10/2014 | 8 | y = 35.48 + 0.38x | 0.83** | 5.06 |
| 14/10/2014 | 9 | ns | ns | 9.60 |
| 19/10/2014 | 10 | ns | ns | 13.53 |
| 25/10/2014 | 11 | y = 60.65 – 4.38x | 0.95** | 8.98 |

r² - Coefficient of determination.

CV - Coefficient of variation.

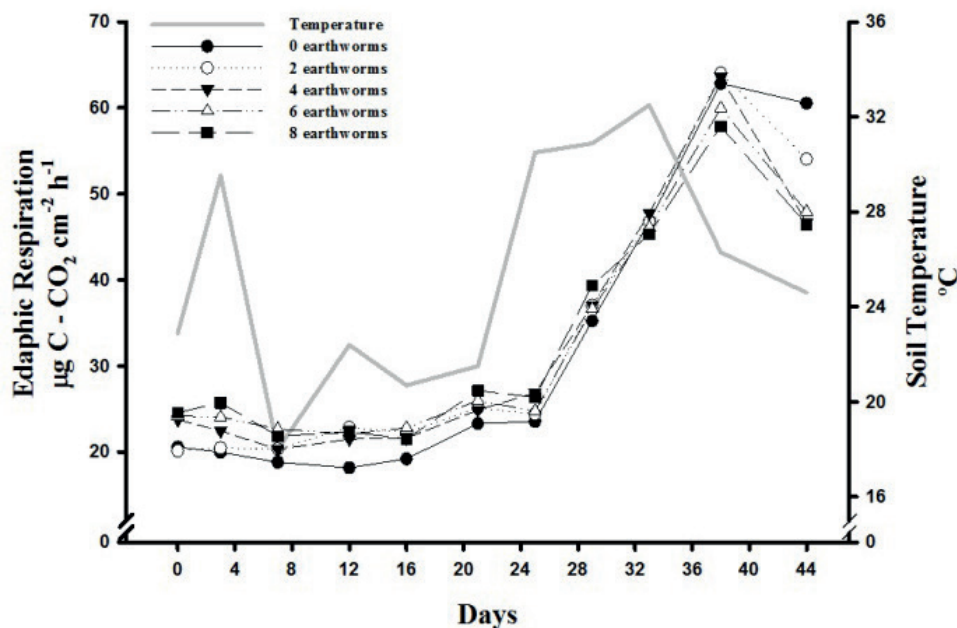
**, * Significance at p < 0.01 and p < 0.05, respectively.

ns - non-significant.

The increased ER by the earthworms remained over 32 days (1st to 8th reading) (Figure 1), after which an inversion in the relationship occurs. After this, a significant difference (p < 0.05) was only observed at the 11th reading (p < 0.01), when the increased number of earthworms resulted in lower CO₂ values. The action of the worms is important for the formation of soil aggregates (MARHAN

et al., 2007), which protect organic material from attack by microorganisms (SOLLINS et al., 1996). Aggregate formation occurs due to cast production, whereby a soil mass passes through the digestive tract of the earthworms (BOSSUYT et al., 2005), especially *Amyntas* spp., which then deposit these casts inside the soil (SNYDER et al., 2009).

Figure 1. Edaphic respiration by earthworm number in each pot (*Amyntas* spp.) and nematodes (*Meloidogyne javanica*) on tomato crops (var. Santa Cruz Kada Paulista).



The remaining proportion of earthworms (39%) at the end of the experiment was less than that found by Stephens and Davoren (1997) grown in a red-brown earth soil artificially infested with *R. solani*, was examined. In soil artificially infested with *R. solani* on wheat chaff, the presence of *A. trapezoides* (at a number equivalent to 300 m⁻² and Du et al. (2014), who found values above 70%. These values occurred because of the escape and/or death of earthworms during the experiment, probably between the 8th and 11th readings, which occurred when the air temperature exceeded 30°C. The optimal temperature for *A. gracilis* survival ranged from 21 to 26°C (SELDEN et al., 2005). In the present study, the soil moisture was adjusted daily, and the soil temperature was the limiting factor for earthworm survival. The accumulated soil respiration (Figure 2a) did not differ statistically between treatments ($p < 0.05$).

The MBC showed quadratic adjustment (Figure 2b) depending on the number of earthworms added to the soil. The lowest and highest MBC ($\mu\text{g C g}^{-1}$ soil) values were 748.5 and 1582.8 $\mu\text{g C g}^{-1}$ soil

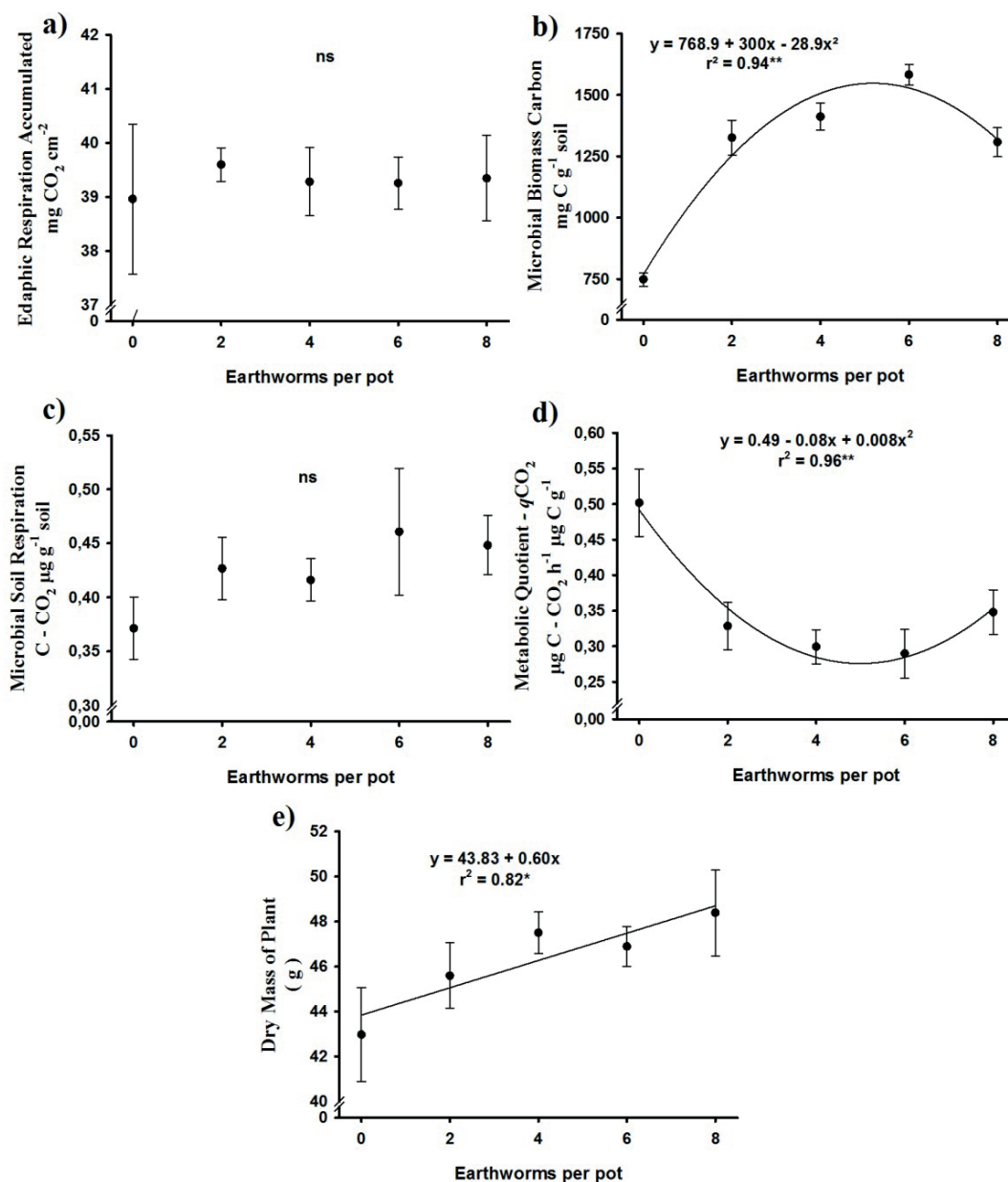
for T4 and T1, respectively. Regression analysis showed that the maximum MBC, 1547.4 $\mu\text{g C g}^{-1}$ soil, occurred with 5.2 earthworms per EU, after which the MBC decreased due to elevated earthworm population.

Inoculation with two earthworms per EU (T1) caused the MBC to increase by more than 75% when compared to treatment without the addition of earthworms (T0). This was also observed by Burtelow et al. (1998) common in many forests of the southeastern US, are invading new habitats in north of their reported range in the northeastern US. At the Cary Arboretum in Millbrook, NY (approximately 42° N latitude, 74° W longitude) who evaluated the invasion of *Aporrectodea* sp. in forests, and found that low densities (50-100 individuals m⁻²) caused a 1.4-fold increase in the MBC when compared with the sites without earthworms. Results similar to those obtained with the T4 treatment (six earthworms per pot) were reported by Al-Maliki and Scullion (2013) by inoculation with *Lumbricus terrestris* versus the control treatment.

The increased MBC may be due to the increased NH_4^+ concentrations inside the casts, which may be 5-15-times higher than those in the surrounding soil (DECAËNS et al., 1999). These concentrations vary depending on the feeding habits of earthworms (geophagous or detritivores) and the quality of ingested material (AIRA et

al., 2003), which can stimulate and increase the microorganism population. Nitrogen is the main limiting nutrient for the development of microbial biomass (HARTMAN; RICHARDSON, 2013); consequently, the community of microorganisms accompanying the fluctuation of this nutrient (HUANG et al., 2013).

Figure 2. Regression analysis of (a) accumulated edaphic respiration, (b) microbial biomass carbon, (c) microbial soil respiration, (d) metabolic quotient, and (e) dry mass of plants with earthworm number (*Amyntas* spp.) per pot in the presence of *Meloidogyne javanica*. Bars represent standard errors.



******, ***** Significance at $p < 0.01$ and $p < 0.05$, respectively.
ns - non-significant.

Galleries formed by earthworms also represent a good source of nitrogen for the microbial community. Parkin and Berry (1999) observed that the drilosphere (1-2 mm from the wall of the galleries) contained $26.6 \mu\text{g N g}^{-1}$ as NO_3^- , while the adjacent soil contained only $18.8 \mu\text{g N g}^{-1}$. This could be related to the mucus produced by the earthworms, which is rich in sugars and is released during movement (BROWN et al., 2000).

An increase in the MBC by the earthworms was also observed by Li et al. (2002), who attributed this effect to the redistribution of resources at depth, such as organic matter and O_2 , increasing the availability for microorganisms.

In the present study, the increase in MBC was not linear, because the T5 treatment (eight earthworms per EU) resulted in values similar to those observed with the T2 treatment (two earthworms per EU), which was 75% higher than those found in the T1 treatment (zero earthworms per EU). This can be explained by the limited resources available in the experimental unit, such as C and N, due to the low availability of soil organic matter for microorganisms, resulting from aggregates formed by the earthworms (MUMMEY et al., 2006). The escape and/or death of the earthworms throughout the experiment due to the high temperature may also have contributed to the results observed for MBC.

The values obtained for MSR (Figure 2c) ranged from 0.37 to $0.46 \mu\text{g C-CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ for treatments T1 and T4, respectively, with no significant differences between the treatments ($p < 0.05$). This is in contrast to the values reported by Binet et al. (1998), who observed increases in excess of 100% following inoculation with *L. terrestris*.

The lack of differences in the concomitant increase in MSR with MBC in the present study, cannot indicate low microbial activity. Increased C-CO_2 evolution by the microbial community may mean that individuals are working in conditions that are not ideal for their metabolism, resulting in a greater loss of soil C to the atmosphere (PÔRTO et

al., 2009). The increase in MBC is often positively correlated with the MSR (BALOTA et al., 1998; PÔRTO et al., 2009). However, D'Andréa et al. (2002) evaluated the impact of management systems on the microbial community, and observed that even with a variation of 200% in MBC between the systems, the MSR remained unchanged, which probably indicated a change in the composition of microorganisms (ANDERSON; DOMSCH, 1993).

Quadratic adjustment was observed for the relationship between MBC and MSR, the metabolic quotient ($q\text{CO}_2$), (Figure 2d) based in the number of earthworms added, with data ranging from 0.20 to $0.50 \text{ mg C-CO}_2 \text{ g}^{-1} \text{ MBC h}^{-1}$ for T4 and T1, respectively. Regression analysis showed a minimum point for $q\text{CO}_2$ of $0.29 \text{ mg C-CO}_2 \text{ g}^{-1} \text{ MBC h}^{-1}$ in response to five *Amyntas* spp. per EU, after which this parameter increases as a function of the higher number of earthworms, confirming the reduction in the MBC.

The data obtained in the present study show that the T4 treatment (six earthworms per EU) increased the MBC while maintaining or reducing metabolic activity, indicating that the presence of earthworms did not affect microbial activity. According to Anderson and Domsch (1993), $q\text{CO}_2$ considers the amount of CO_2 emitted by the soil per C unit present in the MBC; therefore, lower values indicate that the microbial community is more efficient at fixing C in their cells, with higher values observed in young systems, which are still in transition or are disturbed. These findings are not consistent with those by Eisenhauer et al. (2011), who evaluated the impact of exotic worm invasion (*Dendrobaena octaedra*, *Lumbricus rubellus*, *Aporrectodea* spp.) and showed that the composition of the soil microbial community was altered.

The $q\text{CO}_2$ behaviour observed in the present study suggests that the earthworms altered the composition of soil microorganisms by stimulating fungal populations; this was also observed by Aira et al. (2007). These microorganisms represent the

major component of microbial biomass, because of their morphological structure, with long hyphae and large diameter (MOREIRA; SIQUEIRA, 2006). Thus, the soil carbon may be used for the formation of cellular material, building up in the microbial biomass.

The DMP responded positively to increased levels of earthworms (Figure 2e). The highest value obtained for DMP was 48.38 g plant⁻¹, which was observed in the T5 treatment (eight earthworms per EU), and the lowest value was 42.97 g plant⁻¹ with the control treatment (zero earthworms per EU). This represented an increase of 11.2%, resulting from improvements in the chemical, physical, and biological properties of soil promoted by the action of earthworms (BROWN et al., 2006).

Worms lead to physical changes in soil as a result of the galleries, which are commonly used in the growth of plant roots and facilitate water infiltration (YVAN et al., 2012) it is often claimed that earthworm activity could alleviate soil compaction in these systems. To put this assumption to the test, an experimental compaction event was carried out on one plot of arable land. The abundance and biomass of earthworms were evaluated in compacted (under wheel tracks). Chemical changes in the presence of earthworms may have influenced the DMP, due to the increased availability of nutrients, particularly P and N, through accelerated nutrient cycling. Parkin and Berry (1999) reported that the continuous deposition of NH₄⁺ through the action of earthworms

with cast production, stimulates nitrifying bacteria, increasing the availability of nitrogen to plants.

Physico-chemical changes alter the biological components of soil, mainly by stimulating microorganisms (BROWN et al., 2000), which can be reflected in the colonisation of roots by mycorrhizal fungi (AGHABABAEI et al., 2014) and increased nutrient absorption, especially phosphorus. Other effects include the development of plant growth-promoting bacteria (LI et al., 2002), such as *Pseudomonas* spp., which produce siderophores and increase the availability of Fe²⁺ to the plants (COELHO et al., 2007); or in the production of antibiotics, which inhibit the effects of clinical and subclinical pathogens (FREITAS, 2007).

There was a positive correlation between MBC and DMP (Table 2), reinforcing the importance of MBC in plant development. Similar results were observed by De Graaff et al. (2006), who attributed this effect to accelerated nutrient cycling, increasing the availability to plants. There was a negative correlation between qCO_2 and MBC. These results indicate that greater CO₂ was lost from the system with the lower MBC, probably due to stress preventing the full development of microorganisms, and resulting in higher metabolic activity (GRAHAM et al., 2002). In addition, a negative correlation between qCO_2 and DMP suggests that the stress observed in the MBC may also affect plant development (SILVA JÚNIOR et al., 2006).

Table 2. Pearson's correlations among microbial soil respiration (MSR), microbial biomass carbon (MBC), dry mass of plants (DMP), dry mass of roots (DMR), edaphic respiration accumulated (ERA), and metabolic quotient (qCO_2).

| Variables | MSR | MBC | DMP | DMR | ERA | qCO_2 |
|-----------|------|-------|-------|-------|-------|---------|
| MSR | 1.00 | -0.25 | -0.13 | -0.04 | -0.03 | 0.36 |
| MBC | | 1.00 | 0.44* | 0.18 | -0.14 | -0.78** |
| DMP | | | 1.00 | 0.25 | -0.13 | -0.56 |
| DMR | | | | 1.00 | -0.12 | -0.22 |
| ERA | | | | | 1.00 | 0.02 |

**, * Significance at $p < 0.01$ and $p < 0.05$, respectively.

The number of earthworms used in the DMP varied, and was not sufficient to reach the inflection point of the curve, making it impossible to determine the maximum number that can be inoculated in the EUs to maximise production. However, high earthworm densities can lead to overpopulation, which has negative effects on the physical characteristics of the soil. This effect was described by Barros et al. (2004), who evaluated the impact of the mass invasion of *Pontoscolex corethrurus* in the Central Amazon, and observed that high densities of these earthworms increased the topsoil density and

negatively affected plant growth.

The DMR did not differ between treatments (Table 3) ($p < 0.05$), with average values of 4.38 and 4.36 g obtained for the T1 and T5 treatments, respectively. The effects of the earthworms on root development are not yet fully known. Laossi et al. (2010) found that earthworms had similar effects during root development in legumes. Those authors also observed that a positive effect, namely the stimulation root growth, occurred in soils with low fertility.

Table 3. Regression analysis among earthworm number (*Amyntas* spp.), dry mass of plants (DMP)/dry mass of roots (DMR) ratio, and gall number of *Meloidogyne javanica* on tomato crop.

| Treatments (worms per pot) | DMP g plant ⁻¹ | Ratio DMP/DMR | Galls n° |
|-------------------------------|------------------------------|---------------------|--------------------|
| T1 (0) | 4.38 ^{ns} | 8.85 ^{ns} | 1030 ^{ns} |
| T2 (2) | 4.38 ^{ns} | 9.49 ^{ns} | 1006 ^{ns} |
| T3 (4) | 5.07 ^{ns} | 8.57 ^{ns} | 1152 ^{ns} |
| T4 (6) | 4.61 ^{ns} | 9.19 ^{ns} | 1158 ^{ns} |
| T5 (8) | 4.36 ^{ns} | 10.27 ^{ns} | 1021 ^{ns} |
| CV (%) | 11.76 | 14.13 | 15.09 |

ns - non-significant.

There were no significant differences in the ratio among the DMP and the DMR, with the values varying between 8.57 and 10.27 (Table 3). This may be explained by the physiological response of plants, since resources, water, and nutrients are not limited and there is no need for further development of the root system (BELL; SULTAN, 1999).

There was no significant difference in the number of galls between treatments, demonstrating that the plant-parasitic nematode, *M. javanica*, has a high capacity to parasitise the tomato roots. Under the conditions of the present study (earthworms *Amyntas* spp., tomato plants var. Santa Cruz Kada Paulista, phytonematode *M. javanica*), the earthworms were unable to suppress the population of nematodes that were inoculated in the soil.

The failure of earthworms to suppress nematodes, which was determined by the number of galls in the roots, can be interpreted in two different ways: feeding selectivity or inefficiency of the digestive system of worms. Nematodes, unlike fungi, are not considered an essential component of the earthworm diet (BONKOWSKI et al., 2000). Even when nematodes (or eggs and juveniles) are consumed, they may not be digested, because of the low efficiency of the earthworm digestive system (EDWARDS; BOHLEN, 1996), and instead remain alive within the casts.

The low number of worms detected at the end of the experiment (91 days) did not affect the formation of galls, because *M. javanica* infection occurs during the first 7 days after inoculation with the

plant-parasitic nematode (CORTADA et al., 2008). Several authors have reported conflicting results regarding the effect of earthworms on the nematode population (DIONÍSIO et al., 2014; DOMÍNGUEZ et al., 2003; LAFONT et al., 2007; ILIEVA-MAKULEC; MAKULEC, 2002). Therefore, this interaction remains poorly understood, and varies with soil type, and with the plant, nematode, and earthworm species.

Although the number galls was not reduced, decreased stress following nematode attachment to the plants was observed, as demonstrated through the DMP (Figure 7), indicating that the presence of earthworms in the soil was able to compensate for part of the damage caused by *M. javanica*. Lafont et al. (2007) observed a similar effect of *Pontoscolex corethrurus* on the growth of banana plants contaminated by burrowing nematodes (*Radopholus similis*), and attributed this effect to increased nitrogen bioavailability and greater uptake by plants, along with greater absorption of other elements, such as Ca and Mg.

Conclusions

Under the experimental conditions evaluated, inoculation with earthworms of the *Amyntas* spp. was not able to suppress gall formation by nematodes in tomato crop.

The dry matter yield of tomato crop is favoured following inoculating with *Amyntas* spp. and independent of gall formation by the plant parasitic nematode *M. javanica*.

The microbial biomass carbon and metabolic quotient of soil are favoured by the action of *Amyntas* spp. in the presence of the phytoparasitic nematode *M. javanica*.

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