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Research Article

Contribution of organic amendments to soil properties and survival of *Stenocarpella* on maize stalk

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ABSTRACT: The incorporation of organic matter to the soil not only improves nutrient content, but also reduces the survival of *Stenocarpella*, the causal agent of stalk rot, Diplodia ear rot, and grey leaf spot, in maize stubble. We evaluated the effect of organic waste incorporation on *Stenocarpella* survival in maize stalks, the activity of suppressiveness-related enzymes, and nutrient contents. We conducted the assays in the municipalities of Lavras and Sete Lagoas, Minas Gerais State, Brazil. Maize stalks infested with *Stenocarpella* were kept in field conditions for three months after poultry litter, swine manure, fish hydrolysate, compost sewage sludge, and urea application. Infested stalks, without residue amendment, were kept on surface or incorporated into the soil, representing negative and positive controls. *Stenocarpella* concentration in stalks was assessed using qPCR expressed as cycle threshold number. Sewage sludge, buried stalks, and stalks retained on the surface showed reduction of pathogen inoculum. Swine manure and urea did not reduce the quantity of DNA. In the experiment conducted in Lavras, poultry litter stimulated β-glucosidase, urease and hydrolysis of fluorescein diacetate activities when compared to the negative control. Sewage sludge, poultry litter, and swine manure increased Ca in the soil by 44 %, 38 % and 36 %, respectively, in the experiment conducted in Lavras. Poultry litter increased N_{total} three months after application. The results indicate that organic wastes are promising in improving nutrient content, activity of hydrolysis-related enzymes, but *Stenocarpella* inoculum dynamics should be taken into consideration when deciding on the specific organic amendment.

Keywords: *Stenocarpella maydis*, *Stenocarpella macrospora*, *Zea mays*, stalk rot, organic matter

Introduction

No-tillage favors pathogens that survive in maize (*Zea mays* L.) stubbles, such as *Stenocarpella* (Casa et al., 2006). *Stenocarpella* survives in stubble, which may increase the inoculum potential in no-till system causing severe damage. These pathogens have maize as the single host, thus, crop rotation is important to reduce the initial inoculum (Casa et al., 2006). The detection of *Stenocarpella* in the field and the adoption of measures to prevent its establishment in stubbles becomes necessary. Crop rotation reduces initial inoculum of *Stenocarpella* (Flett et al., 2001); however, it requires a long time to reach a suppression condition. Strategies should be developed to increase the contribution of crop rotation to reduce *Stenocarpella* survival.

The introduction of organic matter to stimulate microbial activity in the soil (Bastida et al., 2008) and in maize stubbles could be an important strategy to reduce initial inoculum of *Stenocarpella*. Studies on soil organic matter dynamics have been performed with the objective of controlling plant diseases in an efficient and sustainable way (Bonamoni et al., 2010). Organic matter has been used as a strategy for the management of soil plant pathogens based on its ability to induce soil suppressivity (Hoitink and Fahy, 1986; Lazarovitz et al., 2009). Organic matter incorporation must be studied for each pathosystem. Bonanomi et al. (2007) found that organic matter amendments were suppressive at 45 % of the study, disease incidence increased at 20 %, while no significant effects were verified at 35 %. Thus, the incorporation of organic matter may be an alternative for the control of *Stenocarpella*, however, further studies are needed to prove this effect.

Organic waste incorporated into the soil supplies carbon, nitrogen, and macro- and micronutrients, changes the soil pH and electrical conductivity, increases microbial biomass and enzymatic activities (Lazarovitz et al., 2009; Diacono and Montemurro, 2010; Reddy et al., 2013) could increase the decomposition rate of stubbles, which is associated to the amount and diversity of enzymes released into the soil by the microbial community. The beneficial effects of soil suppressiveness in different soil-borne plant pathogens through incorporation of compost sewage sludge, fish hydrolysate, and poultry litter have been reported (Santos and Bettiol, 2003; Lazarovits et al., 2009; Bettiol and Ghini, 2011; Melero-Vara et al., 2011; Borrego-Benjumea et al., 2014; Heck et al., 2019). The effect of organic matter addition to maize on the survival of *Stenocarpella* has not yet been investigated, while its use as nutrient supply is well explored. We evaluated the effect of organic wastes on the release nutrients to the soil, and the activity of suppressiveness-related enzymes on *Stenocarpella* inoculum through DNA quantification.

Materials and Methods

The assays were conducted in the municipalities of Lavras (latitude 21°14′43″ S, longitude 44°59′59″ W, 919 m a.s.l.) and in Sete Lagoas (latitude 19°28′ S, longitude 44°15′ W, 732 m a.s.l.) both in Minas Gerais State, Brazil. The compost of sewage sludge was obtained from Jundiaí Sanitation Company (Compania de Saneamento de Jundiaí, Jundiaí, SP, Brazil). Fish hydrolysate was obtained from Fish Fertilizers Company (Fish Fertil Indústria e Comércio de Fertilizantes Ltda., Estiva Gerbi, SP, Brazil). Swine manure and poultry litter were obtained from the Federal University of Lavras (Universidade Federal de Lavras, Lavras, MG, Brazil). The chemical characteristics of these wastes are presented in Table 1.

Isolate, inoculum production, and obtaining stalks infected with *Stenocarpella*

Stenocarpella maydis (Berk.) Sutton isolate CML 698 was obtained at the Mycological Collection of Department of Plant Pathology of UFLA. *Stenocarpella* was cultivated on sterilized culture medium (40 g of oatmeal, 18 g of agar, 0.2 g of streptomycin, and 1 L of water) for 15 days at 25 ± 2 °C under a 12-h photoperiod (12 h light/12 h dark). A conidial suspension of *Stenocarpella* (10^6 conidia mL^{-1}) obtained by washing plates with 5-mL sterile distilled water was filtered through a triple layer of sterilized cheesecloth to remove hyphal fragments.

The second internode of maize plants (variety DKB 390 VT PRO 2-DEKALB), at 70 days after planting and cultivated under no-tillage system (without fungicides sprayed) was drilled and 1-mL of spore suspension was injected into the wound. Stalks were harvested 30 days after inoculation, cut longitudinally, and evaluated individually. After evaluation, the stalks were divided into two groups, each one consisting of one longitudinal half from each stalk. One group was stored at −18 °C to determine initial inoculum, and the other group was used for field assays. In the experiments, diseased stalks were placed inside a 64 cm diameter plastic ring (experimental plot) installed on the soil surface. The stalks were placed on the soil with their inner part facing the ground. In each ring, eight stalks were deposited.

Table 1 – Chemical characteristics of organic residues utilized in experiments.

Composition	Poultry litter	Swine manure	Sewage sludge	Fish hydrolysate	Urea
N (g kg^{-1})	41.3	40.5	34.8	-	-
P (g kg^{-1})	26.0	64.5	26.7	-	-
K (g kg^{-1})	36.7	18.2	3.6	-	-
Ca (g kg^{-1})	49.4	41.1	20.0	-	-
Mg g kg^{-1})	11.6	28.8	3.4	-	-
S (g kg^{-1})	30.9	29.8	15.5	-	-
Cu (mg kg^{-1})	366.5	200.7	1018.9	-	-
Fe (mg kg^{-1})	4341.5	1915.1	48647.2	-	-
Mn (mg kg^{-1})	743.4	1039.5	873.4	-	-
Zn (mg kg^{-1})	588.2	1956.9	2574.5	-	-
C total (g L^{-1})	-	-	-	92	-
Ca in water (g L^{-1})	-	-	-	11.5	-
N Total (%)	-	-	-	-	46

After the arrangement of stalks in the rings, organic wastes were applied to the soil and stalks surface. Poultry litter, swine manure, and sewage sludge were applied at doses 960 g (Santos et al., 2009), 544 g (Lazarovits et al., 2009), and 21.3 g (Araújo and Bettiol, 2009), respectively, on each 64 cm-diameter plastic rings. In the plots with sewage sludge, 3.86 g of K_2O were also applied. Fish hydrolysate (0.32 mL per ring) and urea [0.32 g/25.5 mL of water per ring (Bellotte et al., 2009)] were sprayed on the soil. As controls, stalks buried at 10 cm depth and stalks kept on the soil were used. Stalks were evaluated at 90 days after application of organic wastes. The experiments were conducted in randomized blocks (four blocks with one repetition per block), and the distance between the rings was 1 m.

Soil chemical properties

Soil samples were collected between 1 h and 90 days after organic wastes applications at depths 0-20 cm from each ring (250 g soil per ring), and evaluated for pH, organic matter, base saturation, sum of bases, cation exchange capacity, and P, K, Ca, Mg, H + Al; and at depths 0-10 cm (100 g) for C and N analyses.

Enzymatic analyses

Soil samples were collected 90 days after organic wastes applications at depths 0-10 cm from each ring (100 g of soil per ring), and evaluated for hydrolysis of fluorescein diacetate (FDA), β-glucosidase, and urease. The FDA hydrolysis was determined according to Boehm and Hoitink (1992), and expressed in µg of fluorescein min^{-1} g^{-1} of dry soil. The β-glucosidase activity was determined using spectrophotometer after incubation 1 g of dry soil for 1 h in 4.0 mL of MUB, PNG (4-Nitrophenyl-β-D-glucopyranoside) buffer and pH 6.0. The activity was expressed in µg p-nitrophenol g^{-1} dry soil h^{-1}, and the amount of released p-nitrophenol g^{-1} dry soil was calculated based on a standard curve made from a stock solution [100 mL water + 1 mL solution of p-nitrophenol (1 g L^{-1})] (Tabatabai, 1982; Eivazi and Tabatabai, 1988). The soil urease activity was determined according to methodology adapted from Tabatabai and Bremner (1972). A soil sample (5 g) was incubated with 9 mL of 50 mM Tris buffer, with subsequent addition of KCl-Ag_2SO_4, and titration with 0.005 M H_2SO_4. The release of NH_4-N g^{-1} dry soil h^{-1} was calculated. The enzymatic activities in each plot were carried out in triplicate.

Evaluation of population dynamics of the *Stenocarpella* spp.

Five stalks from each plot were collected 90 days after organic wastes applications and the presence of the *Stenocarpella* spp. was evaluated in maize tissues.

The samples were kept under refrigeration at –80 °C. Stalks were reduced to 2-cm fragments, then ground in a Thomas Wiley mill with 3-mm sieves (SPLABOR), and 40 mg subsamples were stored at –18 °C for DNA extraction (Köhl et al., 2015).

DNA extraction was performed using the Genomic DNA Purification Wizard® kit (PROMEGA, Madison, WI) following the protocol of the manufacturer's recommendations. The qPCR was performed on a rotor-gene 6500 (Corbett Research, Montlake, Australia) according to the methodology proposed by Xia and Achar (2001) using SYBR Green PCR Kit (Qiagen), and primers P1 and P2 (P1/2 (GTTGGGGGTTTAACGGCACG/GTTGCCTCGGCACAGGCCGG) specific for the genus *Stenocarpella* to detect the presence of fungus in the stalk samples (Barrocas et al., 2012; Siqueira et al., 2014; Xia and Achar, 2001). For each reaction, a 2.0-µL sample of *Stenocarpella* spp. DNA was mixed into the reaction containing 12.5 µL of SYBR, 0.75 µM of each forward and reverse primer, and 9 µL of nuclease-free water totaling a volume of 25 µL. Initial DNA denaturation occurred at initial 95 °C for 3 min, followed by 40 cycles at 94 °C for 30 s, 60 °C for 1 min and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. A 5-fold serial dilution between 20 ng and 2 pg of isolate DNA from *S. maydis* CML 698 was included in each assay for reference. The calculations were performed based on the values derived from the threshold cycle (Ct values) of the initial (stalks kept under refrigeration) and of the final inoculant samples (stalks collected 90 days after soil deposition).

Statistical analysis

All analytical calculations and graphics were performed using the R software, version 3.2.2. Normal data were analyzed by analysis of variance (ANOVA), and the means were compared by the Scott-Knott test ($p < 0.05$).

Results

Effect of organic residues on soil chemical properties

Ca contents available in the soil in the plots with sewage sludge, poultry litter, and swine manure increased 44 %, 38 %, and 36 %, respectively, compared to plots where the stalk was kept in the soil in the assay conducted in Lavras. Poultry litter increased 25 % of the Ca content in the soil in relation to the control assay conducted in Sete Lagoas (Figures 1A and 1B). No changes in other soil attributes were observed (data not shown).

Application of poultry litter and fish hydrolysate increased N_{total} content (Figures 1C and 1D) in both assays. N_{total} concentrations increased by 21 %, and 23 % with the addition of poultry litter, in the assays conducted in Lavras and Sete Lagoas, respectively. Furthermore, fish hydrolysate provided an increase of 21 % and 22 % (Figures 1C and 1D) to the contents of N_{total} of soil in assays conducted in Sete Lagoas and Lavras, respectively.

The C_{total} content increased with the application of poultry litter in the experiment conducted in Sete Lagoas. The other organic wastes added to the soil did not differ from the control in relation to the C content in both assays.

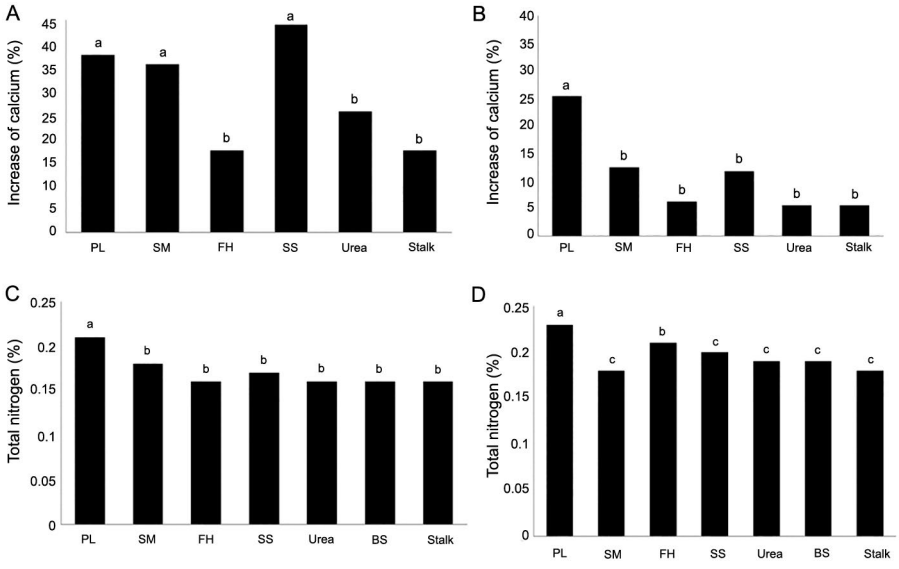

Figure 1 – Effect of poultry litter (PL), compost of sewage sludge (SS), swine manure (SM), fish hydrolysate (FH) and urea on the increase of calcium content (%) in Lavras (A) and Sete Lagoas (B), and total nitrogen (%) Lavras (C) and Sete Lagoas (D) in soil three months after application. BS = buried stalk. Stalk = stalk on the soil surface. Values in each column followed by the same letter do not differ significantly at $p \leq 0.05$ by the Scott-Knott test.

Enzymatic activity in the soil

Poultry litter stimulated the activities of β-glucosidase, urease, and the hydrolysis of FDA in the assay conducted in Lavras (Table 2) in relation to the control (stalk kept on soil surface). In this assay, swine manure, fish hydrolysate, and sewage sludge increased the β-glucosidase activity. In the assay conducted in Sete Lagoas, poultry litter, and urea stimulated β-glucosidase activities (Table 2). However, swine manure, fish hydrolysate, and urea stimulated the FDA hydrolysis similarly to poultry litter (Table 2).

Detection of *Stenocarpella* spp. in maize

Serial dilutions were made with predetermined DNA concentrations of *Stenocarpella* in order to establish a standard curve to evaluate the qPCR efficiency. The standard curve derived from the dilutions of 20 ng at 2 µg of DNA showed an initial efficiency of 127 %, indicating that the assay could be used for DNA quantification. The relationship between DNA copy of the pathogen number and percentage of stalk infection was linear with a high correlation coefficient (R^2 = 0.98) (Figure 2).

The concentration of *Stenocarpella* spp. in the stalk was assessed using the qPCR expressed as the Ct number. The DNA concentration of *Stenocarpella* spp. in plots incorporating sewage sludge, buried stalks, and stalks retained on the surface in the two assays was reduced (Figure 3A). Besides these treatments, poultry litter also reduced the DNA quantity of *Stenocarpella* spp. in maize stalk samples collected in assay conducted in Sete Lagoas (Figure 3B), observed by the increase in Ct. Swine manure and urea did not reduce the quantity of DNA (Figure 3B). In buried stalk, the number of Ct was higher than the stalks kept on the soil, although not statistically different.

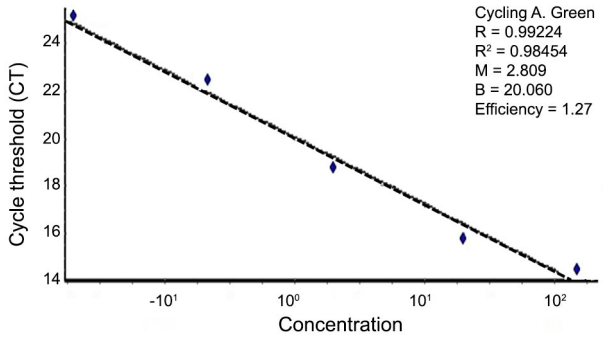

Figure 2 – Linear relationship between DNA concentration and cycle threshold (CT). Slope (M) and Y – Intercept (B).

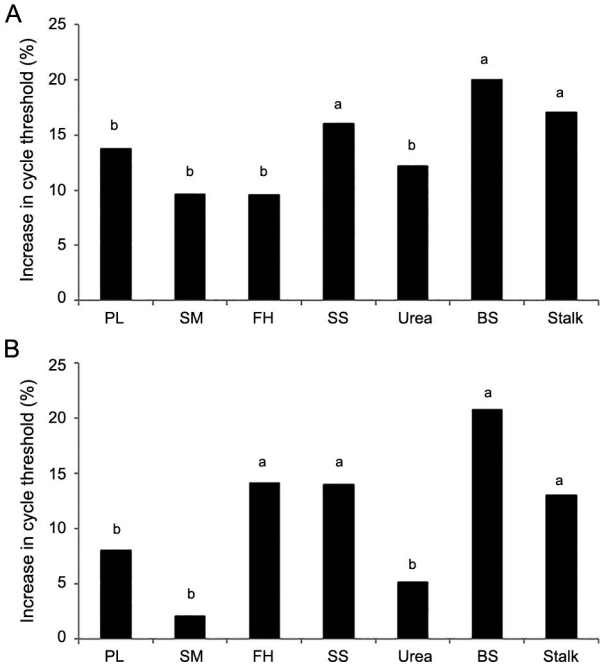

Figure 3 – DNA concentration of *Stenocarpella* spp., in the stalk assessed using qPCR expressed, increased in cycle threshold (%) number in assay conducted at Lavras (A) and Sete Lagoas (B) after three months of application poultry litter (PL), compost of sewage sludge (SS), swine manure (SM), fish hydrolysate (FH), and urea on maize stalk; BS = buried stalk; Stalk = stalk on the soil surface. Values in each column followed by the same letter do not differ significantly at $p \leq 0.05$ by the Scott-Knott test.

Table 2 – Effect of poultry litter, swine manure, fish hydrolysate, compost of sewage sludge and urea incorporated into the soil, and buried stalk and stalk on the soil surface in the β-glycosidase, urease, and hydrolyze of diacetate fluorescein activities in the soil (deep 0-10 cm) in assays conducted in Lavras and Sete Lagoas, Minas Gerais State, Brazil.

Treatment	β-glycosidase p-nitrophenol µg g^{-1} dry soil h^{-1}		µg of FDA hydrolyzed g^{-1}dry soil min^{-1}		Urease µg of N-NH_4 g^{-1} dry soil h^{-1}	
	Lavras	Sete Lagoas	Lavras	Sete Lagoas	Lavras	Sete Lagoas
Poultry litter	14.9 a	14.9 a	1.1 a	0.7 a	159.0 a	162.7 a
Swine manure	12.7 b	10.1 b	0.7 b	0.6 a	55.6 a	144.5 a
Fish hydrolysate	16.3 a	11.3 b	0.7 b	0.5 a	95.4 a	122.6 a
Sewage sludge	15.8 a	7.6 b	0.7 b	0.4 b	83.7 a	124.9 a
Urea	14.0 a	12.8 a	0.9 a	0.5 a	90.2 a	130.6 a
Buried Stalk	9.5 c	9.7 b	0.9 a	0.4 b	162.3 a	126.0 a
Stalk in the soil surface	10.7 c	9.3 b	0.9 a	0.3 b	111.9 a	163.7 a
CV %	10.7	17.2	16.5	23.1	45.1	48.8

Values in each column followed by the same letter do not differ significantly at $p \leq 0.05$ by the Scott-Knott test.

A correlation analysis between the chemical properties and enzymatic activities of soils with the Ct percentage indicated that the FDA hydrolysis correlated positively with the Ct in the experiment conducted in Lavras (R^2 = 0.947; p = 0.05) and in Sete Lagoas (R^2 = 0.917; p = 0.08). The correlation was negative between the Ca content and the Ct percentage (R^2 = -0.458; p = 0.02) for the assay conducted in Sete Lagoas. The correlation analysis between N_{total}, C_{total}, and β-glycosidase and cycle threshold were not significant (Table 3).

Table 3 – Pearson correlation (r) coefficients between β-glycosidase, hydrolysis of fluorescein diacetate (FDA), carbon, total nitrogen, and calcium with cycle threshold (Ct) of DNA concentration of *Stenocarpella* spp. in the stalks.

	Lavras		Sete Lagoas	
	Correlation (r)	*p* value	Correlation (r)	*p* value
β–glycosidase	–0.152	0.375	–0.096	0.576
Hydrolysis of FDA	0.947	0.052*	0.917	0.083
Carbon	–0.265	0.118	–0.068	0.692
Nitrogen	–0.112	0.515	0.125	0.466
Calcium	–0.014	0.521	–0.458	0.024*

*Significant at 0.05 probability level.

Table 4 – Average temperature, rainfall and relative humidity data recorded for Lavras and Sete Lagoas, Minas Gerais, Brazil, during the experiment (June to Aug, 2015).

	Lavras		
	Temperature	Rainfall	Relative humidity
	°C	mm	%
June	18.1	0.0	76.5
July	17.4	0.0	77.0
Aug	18.6	0.0	63.3
	Sete Lagoas		
June	19.2	0.0	66.6
July	19.3	0.0	58.3
Aug	22.0	0.0	54.1

Discussion

The addition of organic wastes and urea to the soil modified its chemical and biological characteristics (Figures 1A, 1B, 1C and 1D, and Table 2). These organic wastes also interfered with the *Sternocarpella* inoculum in maize stalks (Figure 2). The potential of organic residues to change the soil characteristics has been reported in numerous studies (Baker and Cook, 1974; Lazarovits et al., 2009; Bonanomi et al., 2007; 2010; Ghini et al., 2016).

The increase in the number of threshold cycles (Ct) indicates a reduction in the DNA concentration. When quantifying the DNA through qPCR, Ct percentage increased in both stalks kept on the soil and buried, that is, plowing does not affect pathogen inoculum decrease in the short-term.

Summerel and Burgess (1989) suggest that partial incorporation of stubble, for instance by plowing, increases contact between the straw and the soil due to the mechanical breaking of the stalks, favoring their decomposition. Moreover, the increase of stalk contact reduces temperature and humidity fluctuations, favoring microbial activity. However, soil available water and air humidity are of utmost importance for microbial activity (De Leij et al., 1993; Truu et al., 2017). In this study, both assays were carried out in the period with lower humidity (Table 4), which may have impaired the microbial activity (Table 2) and thus, pathogen displacement from stalks.

There was a reduction of *Stenocarpella* spp. in plots where the infected stalks were kept on soil. The reduction on the DNA of *Stenocarpella* spp. in this treatment could be related to several factors, such as the preparation system that keeps a continuous supply of organic matter to soil microorganisms and could act in pathogen suppression. In no-till system, stalks are kept on the soil and often found in this environment until subsequent planting (Saffigna et al., 1989). Plant straws could become a source of energy and nutrients for soil microbial community, favoring the biological activity and enhancing ecological relationships (Powlson et al., 1987; Ferreira et al., 2011).

Reduction of *Sternocarpella* inoculum amended with sewage sludge and fish hydrolysate was observed in the assay conducted in Sete Lagoas. The same reduction was also observed in the assay conducted in Lavras with sewage sludge when compared to urea (Figure 2). None of these treatments resulted in the reduction of *Stenocarpella* spp. inoculum compared to the positive (buried stalk) and negative (stalk on the soil) controls, although the addition of sewage sludge or fish hydrolysate is reported in the control of several soil-borne plant pathogens (Lewis et al., 1992; Lumsdsen et al., 1986; Santos and Bettiol, 2003). The reported suppression is related to the increased soil microbial activity (Lumsden et al., 1986; Lazarovits et al., 2009; Boehm and Hoitink, 1992), which may be due to a positive correlation between the FDA hydrolysis and the increased Ct number, suggesting that the increased microbial activity may contribute to the reduction of the *Stenocarpella* spp. inoculum detected in maize stalks.

The organic wastes, with different chemical characteristics (Table 1), were amended according to the N content and supplied with other nutrients, such as Ca, in different concentrations (Figure 1A). The increase in Ca content is related to the Ca content in wastes, around 20, 49, and 41 g kg^{-1} in sewage sludge, poultry litter, and swine manure, respectively (Table 1). Considering the negative correlation between Ca and Ct (Table 3) observed in the assay conducted in Sete Lagoas, the nutrients may definitively have an impact on the pathogen establishment, as previously demonstrated for *Fusarium graminearum*, another necrotrophic pathogen of maize that is favored by the Mg content buildup in the soil

(Ghini et al., 2016). Ghini et al. (2016), in a study that incorporates two types of sewage sludge into the soil, observed that the incidence of maize stalk rot caused by *Fusarium graminearum* was directly proportional to the content of sewage sludge applied to the soil and with the increasing Mg content. Although nutrients could be limiting factors in stalks (Torma et al., 2017), when supplied at higher amounts, such as those amended in poultry litter, nutrients may lead to *Stenocarpella* inoculum buildup.

Lime and/or superphosphate for disinfection are incorporated where poultry litter is reused for a new batch of hens (Soliman et al., 2018; Vaz et al., 2017). Superphosphate increases the P content of manure, which raises its fertilizing strength. The addition of this mineral could influence the Ca contents in the soil when used as organic manure. After poultry litter and sewage sludge application, the Ca content was changed in the assay conducted in Lavras, according to the soil classification of Alvarez et al. (1999). Other studies showed changes in Ca content when sewage sludge and swine manure were incorporated into the soil (Ghini et al., 2016; Choudhary et al., 1996).

The use of poultry litter increased the N and C contents in soils (Figures 1A, 1B, 1C and 1D), since poultry litter is considered N source. Poultry litter supplied N requirements in maize increasing crop yields (Corrêa et al., 2018). Interestingly, all wastes were amended to supply about the same amount of N. The fact that poultry litter sustained higher N levels in both locations indicated that a more stable form of the nutrient occurs in this organic substrate (Pitta et al., 2012; Corrêa et al., 2018). Besides supplying N to plants, the role of poultry litter on *Stenocarpella* inoculum was not clear and may have even been detrimental, increasing pathogen inoculum. Although poultry litter amendment reduces a soil-borne disease, such as tomato Fusarium wilt (Borrego-Benjumea et al., 2014), it increased the content of *Stenocarpella* sp. inoculum, observed by the reduction in cycle threshold (%), in both locations where it was tested (Figures 3A and 3B), possibly supplying the pathogen with the nutrient that is limiting in such high C:N substrate (Pitta et al., 2012).

Continuous application of organic matter could affect the structure, activities, and microorganism populations producing different enzymes (Hao et al., 2003; Cenciani et al., 2008). We observed an increase in the activity of β-glucosidase and FDA hydrolysis in the soil (Table 2) after wastes application. In both assays, there was increased β-glucosidase activity and FDA hydrolysis after addition of poultry litter or urea (Table 2). In assay conducted in Lavras, there was an increase in these activities for fish hydrolysate and sewage sludge. In the assay conducted in Sete Lagoas, the addition of poultry litter and urea promoted a more efficient enzymatic activity (Table 2). Addition of poultry litter to the soil altered the soil microbial community, which changed the enzymatic activity, inducing suppressivity to soil-borne pathogens. Fish hydrolysate increased β-glucosidase in the assay conducted in Lavras and the FDA hydrolysis in the assay conducted in Sete Lagoas.

Fish hydrolysate may directly control plant pathogens, besides inducing host resistance or stimulating natural biological control agents (Tenuta et al., 2002; Abbasi et al., 2004). We observed that fish hydrolysate did not reduce the survival of *Stenocarpella* spp. in comparison to the controls. However, the survival of *Stenocarpella* was reduced when compared to other treatments such as urea, which is used at the same rate as fish hydrolysate in terms of N load.

The evaluated organic wastes generally showed ability to keep and/or increase the enzymatic activity in the soil, as well as to change nutrient contents, such as Ca, N and C. When the stalks were kept on the soil, buried or in treatments using urea, the enzymatic activity was also increased, demonstrating that the crop system and management types of the cultivated field could alter the activity of soil microbiota, thus, increasing or reducing plant pathogens in these areas.

The results indicate that organic wastes are promising to improve nutrient contents, increasing the activity of hydrolysis-related enzymes, but *Stenocarpella* inoculum dynamics should be taken into consideration when deciding on the specific organic amendment. In general, sewage sludge and fish hydrolysate, compared to positive and negative controls, did not decrease the inoculum of *Stenocarpella* (Figures 3A and 3B). On the other hand, sewage sludge and fish hydrolysate decreased inoculum content compared to poultry litter, swine manure, and urea (Figure 3B). Therefore, the use of sewage sludge and fish hydrolysate to minimize the initial inoculum of *Stenocarpella* in corn crops is more adequate.

In most grain-producing farms in Brazil, in the first season (Oct-Jan) soybean is sown, while in the second season (Jan-Apr), corn is sown. After harvesting, corn stubbles remain on the soil in the no-till cropping system and therefore they serve as a reservoir of necrotrophic pathogen. Thus, in future experiments, we will address such synergistic reduction in the pathogen inoculums of combining both crop management practices, that is, crop rotation and organic wastes incorporation prior to soybean sowing.

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Authors' Contributions

Conceptualization: Bettiol, W.; Medeiros, F.H.V.; Silva, C.A.; Faria, M.R. Data analysis: Faria, M.R.; Guimarães, R.A.; Pinto, F.A.M.F.; Siqueira, C.S.; Silva, C.A. Design of methodology: Medeiros, F.H.V.; Bettiol, W.; Faria, M.R. Writing and editing: Bettiol, W.; Medeiros, F.H.V.; Faria, M.R.

References

Abbasi, P.A.; Conn, K.L.; Lazarovits, G. 2004. Suppression of *Rhizoctonia* and *Pythium* damping-off of and cucumber seedlings by additions of fish hydrolyzed to peat mix or soil. Canadian Journal of Plant Pathology 26: 177-187.

Alvarez, V.V.H.; Novais, R.F.; Barros, N.F.; Cantarutti, R.B.; Lopes, A.S. 1999. Interpretation of soil analysis results = Interpretação dos resultados das análises de solos. p. 314-316. In: Ribeiro, A.C.; Guimarães, P.T.G.; Alvarez, V.H., eds. = Recommendation for the use of correctives and fertilizers in Minas Gerais = Recomendação para o uso de corretivos e fertilizantes em Minas Gerais. 5. Aproximação. Comissão de Fertilidade do Solo do Estado de Minas Gerais, Viçosa, MG, Brazil (in Portuguese).

Araújo, F.F.; Bettiol, W. 2009. Effect of sewage sludge in soil-borne pathogens and powdery mildew severity in soybean. Summa Phytopathologica 35: 184-190 (in Portuguese, with abstract in English).

Baker, K.; Cook, R.J. 1974. Biological Control of Plant Pathogens. W.H. Freeman, San Francisco, CA, USA.

Barrocas, E.N.; Machado, J.C.; Almeida, M.F.; Botelho, L.S.; Pinho, E.V.R.V. 2012. Sensibility of the PCR technique in the detection of *Stenocarpella* sp. associated with maize seeds. Revista Brasileira de Sementes 34: 218-224.

Bastida, F.; Kandeler, E.; Moreno, J.L.; Ros, M.; Garcia, C.; Hernandez, T. 2008. Application of fresh and composted organic wastes modifies structure, size and activity of soil microbial community under semiarid climate. Applied Soil Ecology 40: 318-329.

Bellotte, J.A.M.; Kupper, D.R.; Rinaldo, D.; Souza, A.; Pereira, F.D.; Goes, A. 2009. Acceleration of the decomposition of Sicilian lemon leaves as an auxiliary measure in the control of citrus black spot. Tropical Plant Pathology 34: 71-76.

Bettiol, W.; Ghini, R. 2011. Impacts of sewage sludge in tropical soil: a case study in Brazil. Applied and Environmental Soil Science 2011: article ID 212807. DOI: 10.1155/2011/212807.

Boehm, M.J.; Hoitink, H.A.J. 1992. Sustenance of microbial activity in potting mixes and its impact on severity of Pythium root rot of Poinsettia. Phytopathology 82: 259-264.

Bonanomi, G.; Antignani, V.; Capodilupo, M.; Scala, F. 2010. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. Soil Biology and Biochemistry 42: 136-144.

Bonanomi, G.; Antignani, V.; Pane, C.; Scala, F. 2007. Suppression of soilborne fungal diseases with organic amendments. Journal of Plant Pathology 89: 311-324.

Borrego-Benjumea, A.; Basallote-Ureba, M.J.; Abbasi, P.A.; Lazarovits, G.; Melero-Vara, J.M. 2014. Effects of incubation temperature on the organic amendment-mediated control of *Fusarium* wilt of tomato. Annual of Applied Biology 164: 453-463.

Casa, R.T.; Reis, E.M.; Zambolim, L. 2006. Maize diseases caused by fungi of the genus *Stenocarpella* = Doenças do milho causadas por fungos do gênero *Stenocarpella*. Fitopatologia Brasileira 31: 427-439 (in Portuguese, with abstract in English).

Cenciani, K.; Freitas, S.S.; Critter, S.A.M.; Airoldi, C. 2008. Microbial enzymatic activity and thermal effect in a tropical soil treated with organic materials. Scientia Agricola 65: 674-680.

Choudhary, M.; Bailey, L.D.; Grant, C.A. 1996. Review of the use of swine manure in crop production: effects on yield and composition and on soil and water quality. Waste Management & Research 14: 581-595.

Corrêa, J.C.; Grohskopf, M.A.; Rebellatto, A.; Rigo, A.Z.; Coldebella, A. 2018. Fertilization of high-yield corn with poultry litter based on nitrogen doses. Pesquisa Agropecuária Brasileira 53: 342-350.

De Leij, F.; Dennehy, J.; Kerry, B. 1993. Effect of watering on the distribution of *Verticillium chlamydosporium* in soil and the colonisation of egg masses of *Meloidogyne incognita* by the fungus. Nematologica 39: 250-265.

Diacono, M.; Montemurro, F. 2010. Long-term effects of organic amendments on soil fertility: a review. Agronomy for Sustainable Development 30: 401-422.

Eivazi, F.; Tabatabai, M.A. 1988. Glucosidases and galactosidases in soils. Soil Biology & Biochemistry 20: 601-606.

Ferreira, E.P.B.; Wendland, A.; Didonet, A.D. 2011. Microbial biomass and enzyme activity of a Cerrado Oxisol under agroecological production system. Bragantia 70: 899-907.

Flett, B.C.; McLaren, N.W.; Wehner, F.C. 2001. Incidence of *Stenocarpella maydis* ear rot of corn under crop rotation systems. Plant Disease 85: 92-94.

Ghini, R.; Fortes, N.L.P.; Navas-Cortés, J.A.; Silva, C.A.; Bettiol, W. 2016. Combined effects of soil biotic and abiotic factors, influenced by sewage sludge incorporation, on the incidence of maize stalk rot. PLoS ONE 11: e0155536.

Hao, X.; Chang, C.; Travis, G.R.; Zhang, F. 2003. Soil carbon and nitrogen response to 25 annual cattle manure applications. Journal of Plant Nutrition and Soil Science 166: 239-245.

Heck, D.W.; Ghini, R.; Bettiol, W. 2019. Deciphering the suppressiveness of banana Fusarium wilt with organic residues. Applied Soil Ecology 138: 47-60. DOI: doi.org/10.1016/j.apsoil.2019.02.021

Hoitink, H.A.J.; Fahy, P.C. 1986. Basis for the control of soilborne plant pathogens with composts. Annual Review of Phytopathology 24: 93-114.

Köhl, J.; Lombaers-van der Plas, C.H.; Moretti, A.; Bandyopadhyay, R.; Somma, S.; Kastelein, P. 2015. Analysis of microbial taxonomical groups present in maize stalks suppressive to colonization by toxigenic *Fusarium* spp.: a strategy for the identification of potential antagonists. Biological Control 83: 20-28.

Lazarovits, G.; Abbasi, P.A.; Conn, K.L.; Hill, J.; Hemmingsen, S.M. 2009. Fish emulsion and liquid swine manure: model systems for development organic amendments as fertilizers with disease suppressive properties. p. 49-67. In: Bettiol, W.; Morandi, M.A.B., eds. Biocontrol of plant diseases: use and perspectives = Biocontrole de doenças de plantas: uso e perspectivas. Embrapa Meio Ambiente, Jaguariúna, SP, Brazil.

Lewis, J.A.; Lumsden, R.D.; Millner, P.D.; Keinath, A.P. 1992. Suppression of damping-off of peas and cotton in the field with composted sewage sludge. Crop Protection 11: 260-266.

Lumsden, R.D.; Millner, P.D.; Lewis, J.A. 1986. Suppression of lettuce drop caused by *Sclerotinia minor* with composed sewage sludge. Plant Disease 70: 197-201.

Melero-Vara, J.M.; López-Herrera, C.J.; Prados-Ligero, A.M.; Vela-Delgado, M.D.; Navas-Becerra, J.A.; Basallote-Ureba, M.J. 2011. Effects of soil amendment with poultry manure on carnation Fusarium wilt in greenhouses in southwest Spain. Crop Protection 30: 970-976.

Pitta, C.S.R.; Adami, P.F.; Pelissari, A.; Assmann, T.S.; Franchin, M.F.; Cassol, L.C.; Sartor, L.R. 2012. Year-round poultry litter decomposition and N, P, K and Ca release. Revista Brasileira de Ciência do Solo 36: 1043-1053.

Powlson, D.S.; Brookes, P.C.; Christensen, B.T. 1987. Measurement of soil microbial biomass provides an indication of changes in total soil organic matter due to straw incorporation. Soil Biology & Biochemistry 19: 159-164.

Reddy, A.P.; Simmons, C.W.; D'Haeseleer, P.; Khudyakov, J.; Burd, H.; Hadi, M.; Simmons, B.A.; Singer, S.W.; Thelen, M.P.; Vander-Gheynst, J.S. 2013. Discovery of microorganisms and enzymes involved in high-solids decomposition of rice straw using metagenomic analyses. PLoS ONE 8: e77985.

Saffigna, P.G.; Powlson, D.S.; Brookes, P.C.; Thomas, G.A. 1989. Influence of sorghum residues and tillage on soil organic matter and soil microbial biomass in an Australian vertisol. Soil Biology & Biochemistry 21: 759-765.

Santos, I.; Bettiol, W. 2003. Effect of sewage sludge on the rot and seedling damping-off of bean plants caused by *Sclerotium rolfsii*. Crop Protection 22: 1093-1097.

Santos, I.; Tomazeli, V.L.; Morales, R.G.F. 2009. Organic residues and soil solarization for the control of common bean diseases caused by *Sclerotium rolfsii* = Resíduos orgânicos e solarização para o controle das doenças do feijoeiro causadas por *Sclerotium rolfsii* p. 211-217. In: Bettiol, W.; Morandi, M.A.B., eds. Biocontrol of plant plant diseases: use and perspectives = Biocontrole de doenças de plantas: uso e perspectivas. Embrapa Meio Ambiente, Jaguariúna, SP, Brazil (in Portuguese).

Siqueira, C.S.; Machado, J.C.; Barrocas, E.N.; Almeida, M.F. 2014 Potential for transmission of *Stenocarpella macrospora* from inoculated seeds to maize plants grown under controlled conditions. Journal of Seed Science 36: 154-161.

Soliman, E.S.; Sallam, N.H.; Abouelhassan, E.M. 2018. Effectiveness of poultry litter amendments on bacterial survival and *Eimeria* oocyst sporulation. Veterinary World 11: 1064-1073.

Summerel, B.A.; Burgess, L. 1989. Decomposition and chemical composition of cereal straw. Soil Biology & Biochemistry 4: 551-559.

Tabatabai, M.A. 1982. Soil enzymes. p. 903-947. In: Page, A.L.; Miller, E.M.; Keeney, D.R., eds. Methods of soil analysis. Part 2. Chemical and microbiological properties. American Society of Agronomy, Madison, WI, USA.

Tabatabai, M.A.; Bremner, J.M. 1972. Assay of urease activity in soils. Soil Biology & Biochemistry 4: 479-487.

Tenuta, M.; Conn, K.L.; Lazarovits, G. 2002. Volatile fatty acids in liquid swine manure can kill microsclerotia of *Verticillium dahliae*. Phytopathology 92: 548-452.

Torma, S.; Vilček, J.; Lošák, T.; Kužel, S.; Martensson, A. 2017. Residual plant nutrients in crop residues: an important resource. Acta Agriculturae Scandinavica 68: 358-366.

Truu, M.; Ostonen, I.; Preem, J.K.; Lõhmus, K.; Nõlvak, H.; Ligi, T.; Rosenvald, K.; Parts, K.; Kupper, P.; Truu, J. 2017. Elevated air humidity changes soil bacterial community structure in the silver birch stand. Frontiers in Microbiology 8: 557. DOI: 10.3389/fmicb.2017.00557

Vaz, C.S.L.; Voss-Rech, D.; Avila, V.S.; Coldebella, A.; Silva, V.S. 2017. Interventions to reduce the bacterial load in recycled broiler litter. Poultry Science 96: 2587-2594.

Xia, Z.; Achar, P.N. 2001. Random amplified polymorphic DNA and polymerase chain reaction markers for the differentiation and detection of *Stenocarpella maydis* in maize seeds. Journal of Phytopathology 149: 35-44.