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Residual effect of transgenic soybean in soil microbiota

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Today, Brazil is the second major soybean (*Glycine max* (L.) producer of the world; however, few studies on the effects of transgenesis in the soil microbiota are completed. This study aims at assessing the residual effect of a transgenic soybean with tolerance to the imidazolinone herbicide group on the soil microbiota. The parameters examined were: the soil basal respiration, microbial biomass carbon (MBC) of the soil, metabolic quotient, spores of arbuscular mycorrhizal fungi and total soil fungal and bacterial biomass using epifluorescence technique in areas with eight years of no-tillage cultivated with non-transgenic (BRS133) and transgenic (BRS245) soybeans, in Londrina and Ponta Grossa - PR, Brazil, in the 2011/2012 growing season. The experiment was conducted in a randomized block design and each treatment with six replications. Results showed a significant increase on the MBC in Londrina soils with the BRS245 soybean, and also a significant increase in the total bacterial biomass in the both Londrina and the Ponta Grossa soil with BRS245 soybean, in contrast to those cultivated with BRS133. The BRS245 transgenic soybean crop changed the soil microbiota mainly by increasing the bacterial biomass, which was estimated by the epifluorescence microscopy method. The epifluorescence technique can be used to estimate soil biomass by fungi and bacteria with success.

Key words: Epifluorescence microscopy method, *Glycine max*, soil bacterial and fungal biomass.

INTRODUCTION

The soil is an extremely important component to the functioning of the terrestrial biosphere, not only for food production, but especially for the maintenance of the quality of the local, regional and global environments (Glanz, 1995).

The soil organic matter (SOM) is considered one of the main parameters in the evaluation of soil quality due to its influence on soil characteristics and responsiveness to management practices (Bayer and Bertol, 1999). One of the factors promptly responding to changes in soil use is the microbial biomass, which constitutes a means for processing all organic materials of the soil, acts as a reservoir of nutrients available to plants, and indicates the changes in the annual supply of organic materials by soils (Kaschuk et al., 2010, 2011).

Important parameters can be computed from the microbial biomass, as the metabolic quotient (qCO_2), which is the proportion of basal respiration (SBR) per microbial biomass carbon (MBC). The qCO_2 quotient indicates the influence of environmental conditions on the

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soil organic carbon. It is expected that stressed soils are presenting higher values of qCO_2 (Kaschuk et al., 2010). The SBR represents the amount of CO_2 released from respiration of soil samples and is a very responsive indicator of waste decomposition and disturbances in the ecosystem. This activity of soil organisms is considered a positive attribute for soil quality (Paul et al., 1999).

Agricultural management influences the size and activity of the soil microbiota (Bernardes and Santos, 2006). The ratio fungi/bacteria have been applied as good indicator of mineralization and immobilization of essential nutrients into soils, especially for N and P. Commonly there are more bacteria in conventional tillage and more fungi in non-tillage systems (Vries et al., 2006).

Microorganisms, especially fungi and bacteria, have been described as the principal degraders of organic matter in the soil and the water. Introducing chemical compounds into these environments means supplying microorganisms with nutrients, particularly C, N, and P (Kaschuk et al., 2010).

Currently, the soil capacity of crop production accounts for 40% of total degradation of the world agricultural lands. Among the sources of degradation is the extensive soil tillage. In Brazil, this extensive tillage increased by about 20% in recent years due to increasing demand for grains by the international market, especially for soybeans (*Glycine max* (L.) Merrill).

In the 2010-2011 cropping season, Brazil was the second soybean producer of the world, with 24.3 million ha of area cultivated with the crop and 75 million tons of grains produced, among which 90% from transgenic seeds (Embrapa, 2012). Transgenic soybean is no more than the result of the need for increased production and reduced costs in farming activities.

Genetic modified (GM) soybean was approved for trading firstly in USA in 1996. In 1997, a cultivar was developed by introducing the *ahas* transgene from *Arabidopsis thaliana*, resistant to herbicides of the imidazolinone group (Souza et al., 2013). It is known that the DNA of transgenic plants obtained from exudates, senescence, or degradation of plants or pollen are persistent in the soil (Lunch et al., 2004). Thence, it is important to investigate and understand the effects of transgenic crops on the soil microbiota (Kawalchuk et al., 2003; Dunfield and Germida, 2004; Widmer, 2007).

The BRS245 transgenic soybean, tolerant to herbicides of the imidazolinone group, received the *csr1-2* transgene in its constitution, which codes for the Acetohydroxyacid Synthase (AHAS) (EC 4.1.3.18), the first enzyme leading to the biosynthesis of valine, leucine, and isoleucine (first step of the synthesis of branched-chain amino acids) in plants and microorganisms (Souza et al., 2013). The inhibition of *ahas* activity leads to cell death by disabling cells to produce amino acids essential for protein synthesis and other key metabolic pathways (Souza et al., 2013).

Knowing the impact of transgenic crops and technologies associated with them on environment is an obligation of scientists, government and society. Most of studies on GM plants have assessed their potential only above soil; studies investigating their effects into soil are scarce (O'Callaghan and Glare, 2001; Bruinsma et al., 2003; Kowalchuk et al., 2003; Dunfield and Germida, 2004; Souza et al., 2013). GM plants can change soil microbiota by releasing several substances (exudates), However, the review by Bruinsma et al. (2003) does not address significant effects of transgenic plants on the bacterial and fungal population and microbial respiration of the soil.

On this way, it is important to understand the microbial ecology and soil quality aspects, since microorganisms act in the decomposition of organic matter, directly participating in biogeochemical cycles of nutrients and, consequently, contributing to the availability of nutrients in the soil (Balota et al., 2003; Kaschuk et al., 2010, 2011). Therefore, the soil microbial biomass is an important component of the SOM, responsible for regulating the microbiological transformations and nutrient storage.

Recently, both the soil microbial biomass and its biochemical processes are used as indicators of soil quality by responding promptly to changes in the soil environment. The contents of carbon (C), nitrogen (N) and phosphorus (P) on the soil microbial biomass as well as the activity of microorganisms are very important to understand the nutrient flow in natural ecosystems (Kaschuk et al., 2010, 2011). In addition, plant roots release exudates that can affect the soil microbiota (Koranda et al., 2011), and such changes may occur due to exudation of proteins by transgenic plants (Dunfield and Germida, 2004; Souza et al., 2013).

Many GM plants that are marketed can benefit from mutual associations with microorganisms. Soybean, for instance, form root symbioses with two different groups of mutualistic micro-organisms: bacteria of the genus *Bradyrhizobium*, which fix atmospheric N, and arbuscular mycorrhizal fungi (AMF), which can improve the absorption of nutrients and water by the roots of host plants (Powell et al., 2007).

In recent years, there has largely been cultivation of transgenic soybean in Brazil and in other countries of the southern hemisphere. However, little is known about the impact of GM crops on soil microbial populations and on related parameters, especially under cropping conditions (Souza et al., 2013).

This work aims at assessing the residual effect of the *ahas* transgene on soil microbiota by quantitative analysis, using non-transgenic (BRS133) and transgenic (BRS245 – transgenic version of the parent BR 133) soybeans in different areas, all with eight years of non-tillage (NT) system (Londrina and Ponta Grossa, Paraná, Brazil - 2011-2012 cropping season. Soil basal respiration (SBR), microbial biomass carbon (MBC) of the soil, metabolic quotient (qCO_2), spores density of

Table 1. Soil and local characteristics.

Place	Latitude (S)	Longitude (W)	Climate Koeppen's classification	U.S. soil classification	Previous crop
Ponta Grossa	25° 09´	50° 09´	Cfb	Rhodic Eutrudox	Soybean (summer) / wheat (winter)
Londrina	23° 18´	51° 09´	Cfa	Typic Haplustox	Soybean (summer) / wheat (winter)

arbuscular mycorrhizal fungi, and total fungal and bacterial biomass of the soil were the parameters examined.

MATERIALS AND METHODS

Description of sampling sites and soil sampling

Soil samples were collected in two areas (Londrina and Ponta Grossa, PR – Brazil) under eight years of NT system planted with non-transgenic (BRS133) and transgenic (BRS245 – transgenic version of the parent BRS133) soybeans, the transgenic seeds containing the *ahas* transgene, resistant to herbicides of the imidazolinone group. Soil samples were collected and provided by EMPBRAPA Soybean Division, Londrina - PR, which is a Brazilian experimental station certified to conduct experiments with transgenic material. Table 1 shows the main characteristics of soils and sites of each experimental treatment.

All farming practices as fertilization, irrigation, and pest and disease control were carefully standardized and addressed to each experimental site, following the recommendations for growing soybeans according to each region. The imidazolinone herbicide used was imazapyr at a rate of 70 g a.i. (active ingredient) ha^{-1} and the conventional herbicide consisted of a mixture of bentazon (400 g L^{-1}) + acifluorfen (170 g L^{-1}) (Volt) (570 g a.i. ha^{-1}), in addition to other herbicides such as tepraloxydim, according to the level of weed infestation in each area (ranging from 80 to 240 g a.i. ha^{-1}).

The experiments were conducted with four replications in a randomized block design. Plots were built as eight rows with five meters long and 0.5 m between rows, of which the four central lines were used as experimental area.

At R2 (50% of plants in full bloom) four soil subsamples were obtained at 0-10 cm depth and mixed to form a composite sample. This procedure was repeated six times to make the experimental replications, totaling 24 composite samples. Around 200 g soil per replicate were carefully homogenized and cleaned for plant and root debris and then sieved (4 mm sieve opening) and stored in plastic bags at ±4°C.

Determination of the microbial biomass carbon (MBC) in soil

Estimation of the MBC was made according to the fumigation-extraction method proposed by Vance et al. (1987) and Tate et al. (1988). For fumigation procedure, 10 g of soil sample was weighed and added to 1 mL of ethanol-free chloroform at flasks, which were closed and stored in location free from light for 24 h, with temperature ranging 25 to 28°C. After this period, the lids of the flasks were removed in laminar flow cabinet, leaving all the chloroform to evaporate, as proposed by Brookes et al. (1982) and Witt et al. (2000).

For non-fumigated samples, 10 g of soil sample was also weighed. Both the fumigated and the non-fumigated samples of each treatment were 2-time replicated and the means were obtained. Afterwards, C extraction was performed on fumigated and non-fumigated samples by adding them to 50 mL of 0.5 mol L^{-1} solution of potassium sulfate (K_2SO_4) and then, the samples was stirred for 30 min in an orbital shaker at 220 rpm. After decanting for 30 min, the supernatant was transferred to a filter paper to obtain an extract in a 50 ml tube. To determine MBC, 8 mL of extract was added to a 250 mL Erlenmeyer with 2 mL of 0.066 mol L^{-1} K_2Cr2O_7 (potassium dichromate), 10 mL of 95 to 98% H_2SO_4 (sulfuric acid), 5 mL of 85% H_3PO_4 (ortho-phosphoric acid); and after cooling the solution, 70 mL of deionized water was added. To the cold solution was added 4 drops of 1% (C_6H_5) 2NH (diphenylamine) to make up the titration under magnetic stirring using a solution of 0.033 mol L^{-1} (NH_2) 2Fe (SO_4) $26H_2O$ (ammonium ferrous sulfate). At the end of the titration, the color changed from purple to green.

MBC was estimated from extract by the formula MBC = (Cf – Cnf)/Kc, where Cf and Cnf are the C extracted from fumigated and non-fumigated soil samples and Kc is a constant value used for all samples, according to Hungria et al. (2009). The Kc value used in this study was 0.4, as suggested by Kaschuk et al. (2010).

Determination of basal respiration and metabolic quotient of the soil

The soil basal respiration (SBR) was determined according to Jenkinson and Powlson (1976). 30 g soil samples were weighed and stored in 100 mL flasks with each sample added to 10 mL of 1 mol L^{-1} NaOH (sodium hydroxide) within 30 mL flasks, which were transferred to a 500 mL glass jar tightly sealed. Three glass jars containing only 1 mol L^{-1} NaOH were used as a reagent blank (control).

The samples were incubated for 8 days in a free light area with 25 to 28°C temperature. After incubation period, the NaOH flasks were added to 2 mL of 10% $BaCl_2$ (Barium chloride) and 3 drops of phenolphthalein in alcohol solution at 3% for titration of NaOH with 0.5 ml HCl (hydrochloric acid). Titration was made under magnetic stirring, until the solution goes from white to pink color. Then, SBR was estimated according to Hungria et al. (2009). The metabolic quotient of soil (qCO_2) is the SBR and the MBC soil unit ratio Hungria et al. (2009).

Spore density of arbuscular mycorrhizal fungi (AMF)

Spores were extracted from 8 g soil subsamples by the wet sieving mesh methodology, using 0.710 and 0.053 mm sieves as described by Gerdemann and Nicolson (1963). After centrifugation with water (3000 rpm per 3 min) and with sucrose (2000 rpm per 2 min), the supernatant passed through the 0.053 mm wet sieving and was transferred to Petri dishes for spore counting and identification under a stereoscopic microscope (40X).

Determination of the total soil biomass by fungi and bacteria

The total soil biomass by fungi and bacteria was determined according to Bloem and Vos (2004). 6 g soil sample were weighed, ground, suspended in 190 mL of distilled water and stirred in a

Table 2. Chemical properties* (± standard error) of the soils where the field experiments were conducted.

Parameter[a]	Londrina		Ponta Grossa	
	Soybean varieties			
	BR133	BRS245	BR133	BRS245
pH ($CaCl_2$)	5.67±0.08	5.37±0.08	5.26±0.06	5.11±0.06
SOM (g dm^{-3})	24.85±0.79	25.26±1.04	32.17±1.97	31.57±0.39
P (mg dm^{-3})	27.93±1.45	26.96±2.44	18.51±2.56	19.35±2.44
K ($cmol_c$ dm^{-3})	0.56±0.04	0.48±0.05	0.31±0.02	0.30±0.02
Ca ($cmol_c$ dm^{-3})	4.44±0.14	4.18±0.21	3.45±017	3.28±0.11
Mg ($cmol_c$ dm^{-3})	2.72±0.28	2.03±0.18	1.62±0.09	1.50±0.08
S ($cmol_c$ dm^{-3})	0.89±0.17	1.89±0.34	3.06±0.30	3.13±0.13
CEC ($cmol_c$ dm^{-3})	11.73±0.30	11.28±0.24	10.17±0.23	10.10±0.11
BS (%)	65.55±2.14	59.07±2.59	53.26±1.84	50.15±1.73

[a] P = phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; S = Sulphur. * Methods: P, K, Ca and Mg; extracted by resin; S – extracted by resin $Ca(H_2PO_4)_2$ 0.01 mol L^{-1}; SOM (Soil Organic Matter) – Dichromate/colorimetric. Cation Exchange Capacity (CEC); Base Saturation (BS).

turbo-extractor at maximum speed (20,000 min^{-1}) for 1 min. A 9 mL aliquot of the suspension was transferred to a 15 mL centrifuge tube, gently homogenized in 1 mL solution of 37% aldehyde formaldehyde for 10 s, and allowed to stand for 2 min. After the rest period, an aliquot of 12 µL suspension was distributed uniformly over a microscope slide (9 mm diameter; 64 mm^2) previously sanitized and maintained at 50°C for 2 h to dry. Disinfestations of the slides consisted of washing with liquid detergent and 96% ethanol using absorbent paper. After drying, the slides were placed on damp paper towels.

For determination of fungi, 50 µL of freshly prepared solution of "fluorescent brightener 28 (Sigma F3397: Components C40, H42, N12, O10, S2 and Na2; and 1 mg mL^{-1} of 50% ethanol) was pipetted to each slide.

For the determination of bacteria, 50 µL of fluorescent dye dichlorotriazinyl aminofluorescein [5-(4,6-dichlorotriazin-2-yl)] or DTAF solution [2 mg of DTAF dissolved in 10 ml buffer solution of 0.05 M Na_2HPO_4 (7.8 g L^{-1}) and 0.85% NaCl (8.5 g L^{-1}), adjusted to pH 9] was pipetted to each slide.

The stained slides were stored at room temperature in a dark room for two hours. After this incubation period, the stained slides were immersed in distilled water for determination of fungus. For determination of bacteria, slides were dipped in buffer 3 times for 20 min to remove dye excess, and then dried in the dark at room temperature. Then, the slides received one drop of immersion oil, were covered with a slip and sealed with transparent glaze.

The length of fungal hyphae was estimated by epifluorescence microscopy (400x). The observation was made by the method of intersection of the grid, by randomly selecting one hundred sites and counting the presence or absence of fungal hyphae. The total length of hyphae (mg^{-1} of dry sample) and fungal biomass were calculated as described by Bloem and Vos (2004).

The amount of bacteria was estimated on these slides with epifluorescence microscopy (400x), by randomly selecting one hundred sites and counting for the presence of bacteria. The bacterial biomass was calculated according to equations described by Bloem and Vos (2004).

Statistical analysis

For local, Londrina and Ponta Grossa; and soybean varieties, BR133 and BRS245, T-test were done with independent bilateral averages. The interactions among the treatments with the combinations were submitted to an analysis of variance (ANOVA). Prior ANOVA analysis Levene's test were done for homogeneity. The averages were compared by Duncan's test ($p \leq 0.05$), utilizing the statistical program SPSS, version 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

From soil analysis, the pH values obtained were 5.52 in Londrina and 5.18 in Ponta Grossa (Table 2). The difference in pH values is probably due to soil constitution – in Londrina soil is classified as Eutroferric Red Latosol (Typic Haplustox), while in Ponta Grossa as Dystrophic Red Latosol (Rhodic Eutrudox). In Londrina, it was possible to verify pH values in soils cultivated with BRS245 soybeans lower than those of soils planted with BRS133 soybeans.

The nutrient contents and SOM are influenced by the soil management systems. In NT soils the SOM contents may be significantly higher (Kaschuk et al., 2010). The differences related to soil constitution can explain a variant influence on SOM between Londrina and Ponta Grossa soils, as the respective values 25.1 and 31.9 (mg g^{-1}) indicated (Table 2).

The SBR does not vary significantly between BRS133 and BRS245 varieties in both regions (Table 3), indicating that the transgene has no significant effect on the metabolic microbial activity of the soils. The same was verified by Bruinsma et al. (2003) based on their literature review. However, Bohm et al. (2010), who have studied the effect of herbicides recommended for transgenic soybean (glyphosate and imazethapyr) on MBC observed no significant change in this parameter but a significant increase on SBR with the application of these herbicides. The soybean transgene, however, did not result in significant changes in MBC, SBR and qCO_2 within this study.

Table 3. Values of the soil basal respiration (SBR µg C-CO_2 g^{-1} h^{-1}), microbial biomass carbon (MBC µg CO_2 g^{-1}), metabolic quotient (qCO_2 µg CO_2 $µg^{-1}$ C-microbial h^{-1}), arbuscular mycorrhizal fungi spores density (AMF nº g^{-1} of dry soil), total soil biomass by fungi (µg C g^{-1} of dry soil), total soil biomass by bacteria (µg C g^{-1} of dry soil), total soil biomass by fungi + bacteria (F+B) (µg C g^{-1} of dry soil) and the ratio of total biomass of fungi and soil bacteria (F/B) of soybean varieties (BRS133 – conventional and BRS245 – transgenic) grown in Londrina and Ponta Grossa, PR, in 8 years of non-tillage, in the 2011-12 growing season.

Parameter* / Treatments	SBR	MBC	qCO_2	AMF spores	Fungi	Bacteria	F+B	F/B
Local								
Londrina (LD)	0.75±0.03	139.88±4.40	5.43±0.37	2.79±0.25	23.32±1.60	158.64±12.44	181.96±12.94	0.13±0.01
Ponta Grossa (PG)	0.76±0.03	94.55±3.67	8.17±0.49	4.43±0.32	12.85±0.95	124.81±7.46	137.67±7.81	0.11±0.01
P value	0.841	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.029**	**0.008**	**0.009**
Soybean varieties								
BR133	0.78±0.03	110.18±7.25	7.47±0.58	3.26±0.39	17.55±1.98	117.11±7.13	134.66±7.99	0.15±0.02
BRS245	0.72±0.03	124.26±8.04	6.13±0.51	3.96±0.33	18.63±2.11	166.34±10.07	184.97±11.78	0.11±0.01
P value	0.195	0.207	0.097	0.182	0.712	**<0.001**	**0.002**	**0.032**
Soybean varieties X Local								
BR133 in LD	0.79±0.03	130.34±5.37^{b}	6.13±0.37^{bc}	2.42±0.22^{c}	22.74±2.11^{a}	125.87±11.59^{b}	148.62±12.08^{b}	0.19±0.02^{a}
BRS245 in LD	0.71±0.05	149.35±4.53^{a}	4.74±0.29^{c}	3.17±0.42^{bc}	23.89±2.59^{a}	191.41±10.82^{a}	215.30±12.10^{a}	0.13±0.01^{b}
BR133 in PG	0.78±0.06	89.93±6.21^{c}	8.81±0.79^{a}	4.10±0.58^{ab}	12.35±1.4^{b}	108.35±7.66^{c}	120.70±7.58^{c}	0.12±0.02^{b}
BRS245 in PG	0.74±0.05	99.18±3.47^{c}	7.53±0.52^{ab}	4.76±0.25^{a}	13.36±1.37^{b}	141.28±8.82^{b}	154.63±9.80^{b}	0.09±0.01^{b}
P value	0.582	**<0.001**	**<0.001**	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

*Means (± standard error). For LD and PG (n=12); BR133 and BRS245 (n=12) were done T-test with bilateral independent averages. In combinations, Duncan's test was used (n = 6). *P* values in bold are significant ($p \leq 0.05$).

Bohm et al. (2007, 2011) did not observe significant difference on MBC between transgenic (BRS244RR) and non-transgenic (BRS154) soybean when studied the effects of application and no application of herbicide. However, in present study, a significant increase on MBC was observed for BRS245 in comparison with BRS133 soybean in Londrina. In soils of Ponta Grossa this difference was not significant and the mean values were lower (Table 3).

The MBC increase is a positive aspect since it is an SOM component related to fertility and agricultural sustainability (Kaschuk et al., 2010, 2011). Similar results were found by Souza et al. (2013), who verified a reduced MBC in Ponta Grossa in comparison with Londrina. Low values of MBC can be explained by root exudates affecting soil microbiota (Widmer, 2007; Koranda et al., 2011) and by specific proteins released by transgenic plants (Dunfield and Germida, 2004). Souza et al. (2008) observed MBC changes in Londrina and Ponta Grossa soils during period of pre-sowing (R2 and R8) when studied transgenic and non-transgenic soybeans resistant to glyphosate. The authors found major changes in MBC in Londrina soil.

Zilli et al. (2007) reported a significant lower value of MBC in glyphosate + imazaquin treatment than in the control and the only glyphosate treatments. This result suggests that imazaquin is the responsible to reduce MBC since the glyphosate treatment had its MBC increased. However, some studies have reported no effect of glyphosate on diversity and soil microbiota and on functional activities as SBR and enzymatic action (Lupwayi et al., 2007; Weaver et al., 2007; Hart et al., 2009; Kremer and Means, 2009). But in other study, the application of glyphosate caused significant reduction on total microbial biomass at soybean

rhizosphere of a soil without previous exposition to glyphosate, by changing the microbial population structure (Lane et al., 2012).

The qCO_2 has been used as a biological indicator of soil equilibrium since more the microbial biomass becomes efficient less C is released as CO_2 by respiration and consequently more C is incorporated into microbial biomass (Anderson and Domsch, 1990). On this way, higher the qCO_2, more disturbed the soil is and less C can be incorporated to microbial biomass, causing reduced efficiency to sequester carbon and keep it as a part of SOM. According to Souza et al. (2006), high values of o qCO_2 are found under environmental stress conditions, where the microbial biomass demands more C for its maintenance. Therefore, the qCO_2 value of Londrina (5.43) indicates a less and significantly stressed soil than that of Ponta Grossa (8.17) (Table 3). The BRS245 soybean crop reduced the qCO_2 of Londrina soil (Table 3). This is a positive aspect since C is being kept within MBC, improving the SOM and contributing to the agricultural system sustainability.

The AMF are great bio-indicators of soil disturbance since they are very responsive to changes as soil fertility and plant species and also readily available (Bruinsma et al., 2003; Kowalchuk et al., 2003). In this study, significant effect of the *ahas* transgene was not observed resistant to imidazolinone herbicides of the BRS245 soybean on the AMF spore density. However, a significant higher spore density was observed in Ponta Grossa soil (4.43 no. g^{-1} dry soil), in contrast to a reduced spore density in Londrina (2.79 no. g^{-1} dry soil) (Table 3). Powell et al. (2007) analyzed nine varieties of transgenic soybeans resistant to glyphosate and did not observe significant effect of the transgene on AMF root colonization and on the number and volume of *Bradyrhizobium japonicum* nodules. Reis et al. (2010) also observed that glyphosate applied either single or in sequence did not affect AMF root colonization in soybeans with no seed treatment or treated with endosulfan + tebuconazole.

In Londrina, most of AMF spores were classified as *Glomus* sp., *Acaulospora* sp., and some as *Scutellospora* sp. (data not presented). In Ponta Grossa, most of AMF spores are *Glomus* sp., *Acaulospora* sp., and some are *Gigaspora* sp. These regional differences can be explained by geographical localization and their climate and soil conditions.

The total soil biomass by fungi and bacteria obtained by epifluorescence microscopy method was significantly higher in Londrina than in Ponta Grossa (Table 3). This confirms the results of high MBC obtained by traditional technique as shown in this study in Londrina. No significant differences were observed on fungal biomass in soils planted with transgenic soybeans. Similar results were reported in the literature review by Bruinsma et al. (2003).

A significant increased bacterial biomass was verified in soil planted with the transgenic soybean (BRS245) in both locals, but mainly in Londrina (Table 3). At both locations, studies were performed using the epifluorescence method for estimating the total biomass of bacteria of the soil, but it was not distinguished what soil bacterial groups were affected. Souza et al. (2013) concluded that the soil microbial population did not change significantly with the presence of *ahas* transgene in soybean, after 3 years of consecutive field analysis. From literature review by Bohm and Rombaldi (2010) it can be concluded that genetic transformation of soybean resistant to glyphosate did not affect soil microbiota and biological nitrogen fixation (BNF), but glyphosate applied in weed control can change the microbial soil population and affect BNF. The information survey by O'Callaghan and Glare (2001) shows that transgenic plants cause changes in soil microbiota and plant-microorganisms associations, including BNF (Souza et al., 2008).

The traditional technique for estimating the MBC with chloroform fumigation (Vance et al. 1987) and the epifluorescence microscopy method for estimating the total soil biomass by fungi and bacteria (Bloem and Vos, 2004) are correlated. The MBC correlates significantly with fungal ($r = 0.714$, $p < 0.001$) and bacterial ($r = 0.650$, $p < 0.001$) biomass. The same is observed by fungi + bacteria ($r = 0.707$, $p < 0.001$). These data indicates that the technique of epifluorescence microscopy is suitable to estimate soil biomass by fungi and bacteria and can be used in the further studies with success.

Kowalchuk et al. (2003), in a survey on the effects of all transgenic crops on soil microbes, states that effects have been generally slight and very small when compared to others sources of variation such as tillage, herbicide application and crop rotation. However, those slight changes in the total diversity of the soil microbiota caused by transgenic crops, such as the appearance and disappearance of certain microorganisms (soil bacteria), could affect the ecosystem (Dunfield and Germida, 2004).

Transgenic plants are likely to affect significantly soil populations of non-target bacteria and fungi, but more investigation in a case-by-case basis is needed to a deep assessment of the effects of transgenes on soil microbiota and ecosystems (Liu et al., 2005), as the proposition of this study.

Conclusion

The transgenic soybean crop (BRS245) significantly affected soil microbiota by causing an increased MBC and a reduced qCO_2 in Londrina. An increased total soil biomass by bacteria, obtained by epifluorescence technique, was also observed in soils planted with the transgenic soybean (BRS245) for Londrina and Ponta Grossa. Therefore, the cultivation of transgenic soybean (BRS245) modified the soil microbial population primarily by increasing the soil microbial biomass.

The traditional technique for estimating the MBC using

chloroform and the method for estimating the total biomass of bacteria and fungi are significantly correlated, indicating that the epifluorescence technique can be used to estimate soil biomass by fungi and bacteria with success.

Conflict of Interest

The authors have not declared any conflict of interest.

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