Fulvic acid in foliar spray is more effective than humic acid via soil in improving coffee seedlings growth

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ABSTRACT
Humic (HA) and fulvic (FA) acids improve the nutrient availability and uptake by plants but some aspects of their agronomic use still need to be clarified. The effects of HA soil application and FA foliar application on the growth, Zn and B uptake by coffee seedlings were evaluated. HA was added to an Oxisol at concentrations 0, 10, 25, 50, 75 and 100 mg kg\(^{-1}\) (C-HA), in both limed (pH 6.2) and overlimed (pH 7.2) conditions. FA (0, 0.2, 0.5 and 1 g L\(^{-1}\) C-FA) was applied to coffee leaves in three different application modes (M): with 0.3% Zn and 0.6% B supplied via foliar (M\(_1\)), 0.6% B and 1.2% Zn supplied via foliar (M\(_2\)) and 1.2 mg kg\(^{-1}\) B and 6 mg kg\(^{-1}\) Zn supplied via soil (M\(_3\)). HA addition in soil significantly (\(p < 0.05\)) reduced leaf B and Zn accumulation and coffee growth in both pH conditions. In the M\(_1\) and M\(_2\), FA application significantly (\(p < 0.05\)) increased the shoot growth at 0.59 and 0.45 g L\(^{-1}\) and B accumulation at 0.96 and 0.45 g L\(^{-1}\) C-FA. Foliar application of C-FA, instead soil application of C-HA, is a suitable practice to improve coffee seedlings growth and nutrition on Oxisol.

Introduction
Highly weathered tropical soils are characterized by several chemical restrictions to crop nutrition and growth such as low pH, Al toxicity, low cation exchange capacity (CEC) and reduced availability of macro and micronutrients (Lopes and Guilherme 2016). In low fertility Brazilian soils with the chemical constraints aforementioned, liming and fertilization are routinely applied to many crops, mainly for coffee plantations (Fageria and Baligar 2008; Núñez et al. 2011). In some coffee fields, surface application of excessive lime rates in soils can lead to the overliming condition (pH > 7.0), which decreases micronutrients availability and their uptake by coffee seedlings (Marschner and Rengel 2012). Even at adequate pH, low availability of B and Zn in soil cultivated with coffee is very common and is related to the naturally low levels of both nutrients found in the Brazilian weathered soils (Lopes and Guilherme 2016). Regarding coffee plants, B and Zn nutritional requirements are high, and, in most fields, these nutrients are not efficiently supplied to the plants, hampering coffee plant to reach its potential yield (Martinez et al. 2013; Clemente et al. 2018).

Humic substances (HSs) applications, both via soil or foliar spray, are important strategies to improve soil condition and increase nutrient uptake by plants (Calvo et al. 2014; Canellas and Olivares 2014), both in alkaline (Khaled and Fawy 2011) and in acidic tropical soils with low fertility status (Rose et al. 2014). Combined applications of HS and micronutrients increase their uptake by plants in comparison to the use of the micronutrients alone (Khaled and Fawy 2011; Halpern et al. 2015). Positive effects of HS on plants are related to increased nutrient acquisition and growth as well as...
improved plant resistance to abiotic stress, which are explained by the HS stimulation on plant physiology and biochemical processes (Calvo et al. 2014; Canellas and Olivares 2014). Mechanisms of HS action on plants were throughout reviewed by Canellas et al. (2015, and references therein) and involve changes in the proton pumps, regulation of enzyme activity and levels of plasma membrane proton ATPase (PM H\textsuperscript{+}-ATPase), in a similar way of those effects related to auxins action on plants. PM H\textsuperscript{+}-ATPase enzyme enhances nutrient uptake and increases root growth (Palmgren 2001). The indirect effects of HS are related to the presence in its structure of organic ligands or radicals that can complex micronutrients, keeping them in soluble forms in the soil; HSs also increase the density of negative charges on soil colloids, blocking sites that can strongly adsorb nutrients (Chen et al. 2004; Xu and Peak 2007).

Several soil attributes regulate the magnitude of HS benefits on plants. Soil clay content and levels of C in the soil solution are important to determine the effectiveness of HS effects on crops (Hartz and Bottoms 2010). Soil texture regulates the adsorption of humic materials on clay surface, hampering the direct action of HS on plants (Chotzen et al. 2016). Type and amount of C in soil solution could also act in the same way of exogenous HS added to the soil; thus, a greater response of HS is expected in ecosystems with low levels of dissolved C in the soil liquid phase (Olaetxea et al. 2017) compared to soils rich in readily available C in solution. HS effects on plants are concentration dependent, thus the recommended rates should be carefully studied, because an excess in the soil of HS-derived organic ligands can promote formation of high stable organo-metallic complexes (Boguta and Sokolowska 2016).

Action of HS on plants may also be different due to differences in their molecular weight, presence of plant like-auxins, capacity to complex nutrients and to deliver them to the roots, entrance of humic fragments in root cells, changes in humic structural conformation and chemical nature of organic functional groups linked to humic materials (Canellas et al. 2015; Olaetxea et al. 2017, and references therein). HS chemical and physicochemical properties act regulating the roles played by humic materials on soil and plants. Regarding humic fractions, humic acid (HA) is soluble in aqueous alkaline solutions, but it is precipitated in acidic conditions, and has a higher molecular weight and aromatic character than fulvic acid (FA); FA, on the contrary, is soluble in any soil pH levels due to its higher polarity and has more organic functional groups, negative charge density and hydrophilic radicals than HA (Stevenson 1994).

Variability in responses of crops to HSs is also attributed to the application mode, considering HS is supplied via soil or foliar spray (Olaetxea et al. 2017). Regarding foliar application of HS, accessibility of humic colloids by plant tissues is efficiently increased in relation to HS added via soil (Khaled and Fawy 2011; Olaetxea et al. 2017). In foliar sprays, FA probably has higher agronomic efficiency in comparison to HA due to, among other factors, its higher solubility at low pH media, which is typical of foliar sprays (Fernández et al. 2013). HS concentration required by the effective action on plant metabolism and growth when applied via soil is greater than those rates required when HSs are supplied via foliar spray (Anjun et al. 2011). So, considering that most of the studies investigate soil applications of HS (Calvo et al. 2014), studies with foliar application of HS are important to set adequate concentrations of different molecules regarding this specific application mode.

Plant type is also a factor regulating the extent of HS action on growth, development, uptake and use efficiency of nutrients by crops (Rose et al. 2014; Halpern et al. 2015). Woody perennial plants like coffee are less responsive to humic fertilization than grass and leguminous (Rose et al. 2014), though some studies have reported HS-positive effects on growth and fruit mass and quality for pear, olive and lemon (Calvo et al. 2014). Despite the large consumption of coffee and its economic importance for Brazil and the world, there is no study in which effects of HS both added to soil or via foliar were tested for this crop. The aim of this study was to investigate the effects of HA concentrations added to limed and overlimed soil samples and the influence of foliar FA concentrations and modes of B and Zn application on the availability of both nutrients in soil and in its solution. We also investigated the effects of both HA (soil) and FA (leaf) concentrations on nutrition and growth of coffee seedlings.
Material and methods

Soil, seedlings and HS characterization

Two greenhouse experiments were used to assess the effects of soil applied HA (Experiment I; EI) and foliar applied FA (Experiment II; EII) on coffee seedlings. The experiments were carried out at the Department of Soil Science/Federal University of Lavras, Lavras, Minas Gerais state, Brazil, under 21°14’43” S, 44°59’59” W and altitude of 919 m. A loamy Typic Hapludox soil (Oxisol) from the 0–0.2-m depth was sampled in a tropical native forest located in Lavras, Minas Gerais state, Brazil, and used as growth medium for coffee seedlings evaluated in both experiments. The main soil characteristics under natural vegetation conditions are described as follows: pH: 4.1; N – NH₄⁺: 12 mg kg⁻¹; N – NO₃⁻: 10 mg kg⁻¹; P (Mehlich-1 solution): 1.9 mg kg⁻¹; K⁺: 30 mg kg⁻¹; Mg²⁺: 0.1 cmolc kg⁻¹; Ca²⁺: 0.3 cmolc kg⁻¹; available B: 0.1 mg kg⁻¹; available Zn: 0.4 mg kg⁻¹; Al³⁺: 1.1 cmolc kg⁻¹; H + Al: 9.8 cmolc kg⁻¹; CEC at pH 7: 10.2 cmolc kg⁻¹; base saturation: 5%; Al saturation: 70%; soil organic matter (SOM): 3.2 g kg⁻¹; total N: 2 mg kg⁻¹.

Coffee arabica L. var. Catuaí vermelho (I-144) seedlings used for both experiments were obtained from a commercial nursery located in Nepomuceno (MG State, Brazil). Coffee cultivation was performed with 28 week-seedlings transplanted to pots (190 mm of diameter) with 3 kg of soil with six pairs of leaves completely developed.

Both HA and FA used in this study were commercial products extracted from leonardite, following the extraction and purification methods proposed by Swift (1996). The main properties of humic materials used in this study are described as follow: electrical conductivity (EC): 37.7 dS m⁻¹; pH: 9.7, E4/E6 ratio: 4.84; C: 38%, N: 0.9% for the HA sample; EC: 19.1 dS m⁻¹; pH: 5.6, E4/E6 ratio: 7.35; C: 39%, N: 0.35% for the FA sample.

Experimental conditions and treatments

In the EI, six HA concentrations based on its C content (C-HA: 0, 5, 10, 25, 50 and 100 mg kg⁻¹) were added to the Oxisol limed to reach two pH levels: 6.2, which is the normal soil acidity degree required by coffee plants, and 7.2, a level routinely verified in overlimed soil coffee plantations. Therefore, a 2 × 6 factorial scheme was adopted through the combination of six HA concentrations with two soil pH levels. The treatments were arranged in a randomized block design with four replicates.

In the EII, increased concentrations of FA were applied on coffee shoot in combination with different application modes of B and Zn. Three application modes (M) of Zn and B were used as follows: 0.3% B (H₃BO₃) and 0.6% Zn (ZnSO₄·7H₂O) in a foliar spray (M₁); 0.6% B (H₃BO₃) and 1.2% Zn (ZnSO₄·7H₂O) in a foliar spray (M₂) and 1.2 mg kg⁻¹ B (H₃BO₃) and 6 mg kg⁻¹ Zn (ZnSO₄·7H₂O) supplied in the soil (M₃). For the three application modes of micronutrients, C-FA was supplied via foliar spray at the following concentrations: 0, 0.2, 0.5 and 1 g L⁻¹, whose range was based on Suh et al. (2014). Distilled water was used in the preparation of all the solutions used as foliar sprays. Therefore, a 3 × 4 factorial scheme was adopted through the combination of three application modes of micronutrients with four C-FA concentrations supplied via foliar sprays. The treatments were arranged in a randomized complete block design with three replicates.

In order to reach the two soil pH conditions for EI (6.2 and 7.2) and pH 6.2 (considered ideal for coffee growth) for EII, reagent grade carbonates (CaCO₃:MgCO₃ at 3:1 proportion) were mixed with soil samples (3 kg) and allowed to react for 30 days with soil moisture kept close to 70% of field capacity (FC). Amounts of carbonates used to reach the two soil pH levels tested (13 g kg⁻¹ for pH 6.2 and 21 g kg⁻¹ for pH 7.2) were based on the Oxisol acidity neutralization curve described in Carmo et al. (2016). In EI, limed soil samples were sieved (2 mm) and mixed with increasing C-HA concentrations. Coffee seedlings were carefully taken off from plastic bags of 1 kg, the root was washed with distilled water and, in sequence, plants were transplanted into pots. Only one coffee seedling was cultivated per pot.
Coffee seedlings were fertilized according to nutrient rates adapted from Novais et al. (1991) for plant cultivation in pots, as follows: 400 mg kg\(^{-1}\) N, 400 mg kg\(^{-1}\) K, 40 mg kg\(^{-1}\) S, 1.3 mg kg\(^{-1}\) Cu, 4 mg kg\(^{-1}\) Mn, 0.15 mg kg\(^{-1}\) of Mo. B and Zn were applied at recommended rates by Novais et al. (1991) (1.2 and 6 g kg\(^{-1}\), respectively) only in the EI. In the EII, these micronutrients were supplied according to different application modes described above. Phosphorus fertilization was applied before coffee seedlings were transplanted into pots through the addition of 300 mg kg\(^{-1}\) P (Novais et al. 1991). N and K were split into four topdressing applications in equal amounts of 100 mg kg\(^{-1}\) N and 100 mg kg\(^{-1}\) K each time, at 30, 60 and 90 days after planting (DAP) for EI, and after 35, 65 and 95 DAP for EII. Regarding soil solution extraction, the Suolo Acqua sampler was placed in each experimental pot (Carmo et al. 2016) during the coffee seedlings transplant. After keeping soil moisture near 100% FC for 12 h, aiming at the equilibrium between soil liquid and solid phase, soil solution was sampled for analysis.

In the EII, B and Zn were combined in different ways with increased C-FA concentrations supplied via foliar spray, which were applied on coffee shoot at 30, 60 and 90 DAP. The total volume of foliar sprays containing FA + B + Zn (M\(_1\) and M\(_2\)) or only FA (M\(_3\)) added to each coffee plant was 25 mL. Coffee seedlings were grown for 120 days in the EI and for 105 days in the EII. Soil moisture during the coffee growing was kept close to 70% FC, through the addition of bi-distilled water on a daily basis.

**Soil and plant analysis**

Coffee plants were harvested and separated in leaf and stem and root parts. Dry matter (DM) of all these parts was determined after drying coffee tissues at 60°C for 72 h. Root dry mass (RDM) was divided by shoot dry matter (SDM, stem + leaf) in order to calculate the root-to-shoot DM ratio. B and Zn contents were determined in leaf, stem and root by atomic absorption spectrometry after the plant tissue digestion in a HNO\(_3\)–HClO\(_4\) solution at a 4:1 ratio (Teixeira et al. 2017). B and Zn nutrient contents (mg kg\(^{-1}\)) and DM (g) of the respective plant tissue were used to determine the accumulated nutrient content (mg plant\(^{-1}\)).

Soil solution collected at the beginning of the experiments was analyzed for EC with a conductivity meter Mettler Toledo and pH with a pH/ORP meter HANNA instruments. Zinc in the soil solution was determined at the beginning of coffee cultivation in both experiments using the inductively coupled plasma optical emission spectrometry (Spectro, Blue, Germany) technique (Teixeira et al. 2017). The instrument working conditions were as follows: plasma power 1400 W, plasma, auxiliary and nebulizer gas flow of 12, 0.8 and 0.85 L min\(^{-1}\) and the emission line for Zn was 213.856 nm. C in the soil solution was determined in an Elmental Automated Analyzer (Elementar, VARIO TOC Cube Model, Germany) using the liquid module (Carmo et al. 2016).

**Statistical analysis**

The data set was submitted for analysis of variance (\(p < 0.05\)) through the R computer software version 10. The Tukey test at \(p < 0.05\) was applied to compare differences among treatment means. The regression analysis was also performed to evaluate the influence of HA or FA concentrations on soil and plant attributes evaluated in this study. Principal component analysis (PCA) was performed through the R software (Oksanen et al. 2015) and used to identify clusters or to establish the relative weight of HSs concentration and types (HA or FA), and micronutrient application modes on soil and coffee seedlings nutrition and growth attributes. PCA analysis also allowed the study of the main pattern of data set structure. The normalized distribution of soil and plant attributes over the factors and their levels was studied through analyzing their clustering on the PCA axes.
Results

EI

The initial conditions of soil and soil solution pH, EC, C and B and Zn availability as affected by C-HA concentrations added to the Oxisol are shown in Figures 1 and 2. Interaction between soil pH conditions and C-HA concentrations tested was not observed for soil (Figure 1(a)), soil solution pH (Figure 2(a)) and for EC (Figure 2(b)). C-HA concentrations applied to soil did not influence the initial soil pH values (Figure 1(a)). In soil solution, 50 mg kg$^{-1}$ C-HA slightly raised soil pH levels (Figure 2(a)). Soil solution EC in both limed and overlimed samples linearly increased over C-HA concentrations (Figure 2(b)). In the overlimed soil, EC reached values next to 3.5 dS m$^{-1}$ at the highest C-HA concentration applied (100 mg kg$^{-1}$). C in soil solution was not affected by C-HA addition, with mean values of 290 and 265 mg L$^{-1}$ for overlimed and limed soil conditions, respectively (Figure 2(c)). C in soil solution of the overlimed Oxisol increased as the C-HA concentration is elevated, reaching a plateau for concentrations higher than 50 mg kg$^{-1}$ C-HA.

Interactions between pH levels and C-HA concentrations were observed for Zn and B soil availability (Figure 1(b,c)). In all cases, the quadratic model best adjusted to B and Zn contents over C-HA concentrations. Regarding the overlimed soil (pH 7.2), as C-HA concentration was increased from 25 to 100 mg kg$^{-1}$ C-HA, B also increased, reaching a maximum level in soil at the highest C-HA concentration (Figure 1(b)). Soil Zn content decreased to 5 mg kg$^{-1}$ when C-HA was added at 43.5 mg kg$^{-1}$ (Figure 1(c)). With 100 mg kg$^{-1}$ C-HA, soil Zn levels in the overlimed soil were 14% higher than Zn levels in the HA control soil (Figure 1(c)). In soil solution from the overlimed soil, the highest Zn content was observed at 50 mg kg$^{-1}$ C-HA (Figure 2(d)). On the contrary, in the limed soil (pH 6.2), soil B increased for C-HA concentrations up to 38.5 mg kg$^{-1}$, with a decline in B availability for higher C-HA concentration than the already mentioned (Figure 1(b)). The lowest level of Zn in the limed soil and in its soil solution was verified for 59.4 and 50 mg kg$^{-1}$ C-HA, respectively (Figures 1(c) and 2(d)).

The influence of C-HA and soil pH levels on coffee seedlings biomass is shown in Figure 3(a–c). Coffee seedlings DM was not influenced by the interaction between soil pH levels and C-HA concentrations. In all cases, the quadratic equation was the best model that fitted to the data set ($R^2 = 0.98–0.99$). All coffee compartments analyzed presented the lowest biomass when C-HA was added to soil at concentrations around 50 mg kg$^{-1}$. Biomass production verified for application of 100 mg kg$^{-1}$ C-HA in soil was higher than that verified for the HA control soil only for root growth (Figure 3(a–c)). DM for each plant compartment was higher when coffee was cultivated in the limed (pH 6.2) than in the overlimed soil (pH 7.2) (Figure 3(a–c)). Regardless of the soil pH, root-to-shoot DM ratio was linearly increased over C-HA concentrations (Figure 3(d)).

Figure 1. Influence of C-HA concentrations on initial soil pH (a), soil available B (b) and soil available Zn (c). Mean with the same letter does not differ by the Tukey test at p < 0.05. LSD: The least significance difference based on the Tukey test at p < 0.05.
Amounts of B and Zn accumulated in coffee shoot seedlings over C-HA concentrations and in plants grown in both limed and overlimed soils are shown in Figure 3(e,f). Regarding coffee B uptake, the mathematical equation that best fitted to the data set was the quadratic one and there was no interaction between soil pH levels and C-HA, on B concentration in coffee shoot. The minimal B content in coffee shoot was observed at 53.8 mg kg\(^{-1}\) C-HA for both soil pH conditions (Figure 3(e)).

Zn accumulated in coffee shoot was not changed as the C-HA concentration was increased in the overlimed soil (pH 7.2; Figure 3(f)). In the limed soil (pH 6.2), the lowest amount of Zn accumulated in coffee shoot was verified at 76.5 mg kg\(^{-1}\) C-HA (Figure 3(f)). In the limed soil, a sharp decrease in coffee shoot Zn content is observed as C-HA concentration is increased (Figure 3(f)).
Levels of pH, EC, B and Zn in soil and solution from the Ell as a function of C-FA concentrations applied to coffee shoot and B and Zn application modes are shown in Figure 4. Soil pH, soil solution pH and EC were not affected by micronutrient application modes (Figure 4(a–c)). Increases in contents of B and Zn in soil were verified only when both nutrients were added to soil (M3; Figure 4(d,e)). Levels of Zn in the soil solution did not change as a function of C-FA concentrations, even when both nutrients were mixed with the whole soil (M3; Figure 4(f)).

DM production for each coffee plant compartment and the root-to-SDM ratio as a function of C-FA concentration in foliar spray are shown in Figure 5(a–d). In general, the highest DM values for all plant compartments were verified when B and Zn were applied to leaves, regardless of their concentrations (M1 or M2; Figure 5(a–c)). In fact, only leaf DM is influenced by C-FA addition, with maximum at 0.59 g L⁻¹ C-FA for M1 and 0.45 g L⁻¹ C-FA for M2 (Figure 5(a)). Increases in leaf DM aforementioned were 47% and 19% in relation to leaf DM determined for FA control plants (Figure 5(a)). When C-FA was sprayed on leaves without B and Zn (M3), leaf DM was not changed (Figure 5(a)). DM root-to-shoot ratio was B–Zn application mode dependent. When B and Zn were added directly to the soil (M3), the root-to-shoot ratio decreases linearly over C-FA concentrations (Figure 5(d)). On the contrary, when FA was applied at the highest concentrations of B and Zn in the foliar spray (M2), the quadratic model was the equation that best adjusted to root-to-SDM ratio; the lowest production of root-to-SDM was verified at the 0.52 mg L⁻¹ C-FA (Figure 5(d)). When B and Zn were applied to the leaves at the lowest concentration (M1), the root-to-SDM ratio was not influenced by the increasing C-FA concentrations applied to coffee leaves (Figure 5(d)).
Changes in Zn and B accumulated in coffee seedlings shoot as a function of foliar applied C-FA concentrations are shown in Figure 5(e,f). In the $M_1$ and $M_3$ application modes of micronutrients, Zn accumulated in coffee shoot was not affected by C-FA foliar concentrations (Figure 5(f)).
Regarding the M2 application mode, on the contrary to the leaf DM response to FA application (Figure 5(a)), Zn accumulated in coffee shoot decreased over C-FA concentrations (Figure 5(f)). In another way, the accumulated amounts of B verified for M1 and M2 treatments were maximum at 0.96 and 0.45 mg L\(^{-1}\) C-FA, respectively (Figure 5(e)), following a quadratic model (Figure 5(a)). The best C-FA concentrations already mentioned increased B content by 63% for M1 and 18% for M2. Regarding the B addition to soil (M3), B content in shoot was not affected by the C-FA concentration in foliar spray (Figure 5(e)).

**PCA**

In order to confirm the relationships amongst the different modes of HS application and summarize the experiments results, a PCA for EI and EII was performed (Figure 6). For the EI, the PCA plots (79% of the total variance explained) showed a clustering of most coffee biomass attributes (root and shoot) with negative values in the PC1 (67%), which was directly related to soil limed to reach pH 6.2. Soil overliming is the main factor that negatively affects coffee seedlings growth. The marked increase in soil pH from 6.2 to 7.2 also increased soil EC and soil solution pH to levels beyond those considered optimum for coffee growth. In the EI, the key drive force regulating the magnitude of soil and plant attribute changes, instead of C-HA concentration, it is the level of pH reached in both limed and in the overlimed Oxisol samples (Figure 6(a)).

In the EII (Figure 6(b)), DM components (except root) and B and Zn accumulation are closely associated mainly with the M2 (the left side of the plot) with negative PC2 values. In PC2 axis, RDM, soil solution Zn and EC are closely associated with M1. Boron and Zn supplied via soil (M3) are not suitable strategies to supply both nutrients to the coffee. However, it is a right practice to increase Zn and B contents in soil, though an increase in the availability of both micronutrients did not assure the highest accumulation of B and Zn in coffee seedlings shoot (Figure 6(b)).

**Discussion**

**Soil conditions and B and Zn availability according to HS application**

Micronutrient availability for plant is determined by its content in soil solution, which is in equilibrium with adsorbed and precipitated nutrient forms in the soil solid phase (Marschner and
The main factors affecting the type and amount of precipitated and adsorbed micronutrients in soil are pH, clay and SOM content (Sauvé et al. 2000). Elevation in soil pH (>7), here typically characterized by the soil overlimed condition, reduces the availability of B and Zn, since it increases the adsorption site density in clay minerals, increasing the amount of micronutrients bound into soil colloids (Lemarchand et al. 2005). Furthermore, pH affects the chemical species distribution of nutrients in soil. For B, pH >6.5 increases quickly the amount of the B(OH)\textsuperscript{4−} form, which has greater affinity by mineral surfaces and also organic matter sites than the B(OH)\textsubscript{3} form (Xu and Peak 2007). For Zn, elevation of soil pH (>7) increases insoluble Zn species (Marschner and Rengel 2012). In this way, in the control soils from EI, a reduction of Zn and B content under the overlimed condition compared to levels of B and Zn in limed soil (pH 6.2) was observed (Figures 1(b,c) and 6(a)), probably, due to greater adsorption and precipitation of Zn and B into soil colloids in the overlimed soil condition.

HS fractions with different solubility and reactivity, present and/or added to the soil, and their associations with micronutrients are also processes that change the equilibrium of soluble and insoluble nutrient forms (Keren and Communar 2009). Soluble fractions of organic matter have sites to bind micronutrients, consequently, forming organomineral complexes (Wang and Xing 2004), which may keep nutrient in soil solution even in conditions that promote more precipitation or adsorption as soil pH is increased to levels in alkaline soil pH range (Garcia-Mina et al. 2004). Nevertheless, if there is a great amount of HA/FA in relation to micronutrient, high stability inner-sphere complexes are formed (Boguta and Sokołowska 2016), which decreases the readily available micronutrient species to cell roots. In addition, the association of insoluble HA-metal chelates in soil may decrease levels of micronutrient in soil solution. HS also could bind and/or cover soil minerals surface, blocking potential adsorption sites of micronutrients (Xu and Peak 2007).

Based on the assumptions aforementioned, in the EI, and in the soil with pH 6.2, there is more B predominating in soil solution as B(OH)\textsubscript{3} form than in the soil with pH 7.2. When added to soil at rates up to 38.5 mg kg\textsuperscript{−1} C-HA, organomineral complexes available for plants (low–medium bind energy) could be formed, with subsequent increase of soil available B content (Figure 1(b)). On the other side, under high organic ligand abundance in soil (provided by the highest HA concentration), probably high-energy organomineral complexes are formed and soil B availability was reduced (Figure 1(b)). At the overlimed condition (pH 7.2), increase of soil available B was observed for C-HA concentrations higher than 25 mg kg\textsuperscript{−1} C-HA (Figure 1(b)). Based on the assumption that, at pH 7.2, there is more adsorption of B than in soil with pH 6.2, we could hypothesize that HA has a role in blocking B adsorption sites in the mineral phase, in line with results reported by Xu and Peak (2007). Contrary to B, Zn has much more affinity and, consequently, chemical interaction with colloids in soil mineral phase than with organic ligands (Sauvé et al. 2000). In soil, most Zn predominates in the mineral phase and, in very low levels, in soil solution. This explains the very low Zn contents in soil solution determined in soil of EI (Figure 2(d)) and EII (Figure 4(f)). In the EI, Zn application in the soil was not effective in increasing Zn in soil solution, whose levels were similar to the treatment in which Zn was only applied on a coffee shoot (Figure 4(f)). Regarding HA effects on soil available Zn of EI, in both soil pH conditions, even the addition of 100 mg kg\textsuperscript{−1} HA probably was not enough or ineffective in blocking Zn adsorption sites, in line with results reported by Wang et al. (2016). In addition, the abundance of HS in soil may decrease even more the low levels of Zn remaining in soil solution, due to formation of inner-sphere/insoluble organo-mineral complexes, which reduces the soil Zn availability (Boguta and Sokołowska 2016) (Figure 1(e)). In soil solution of the limed soil (pH 6.2), Zn content showed the same pattern observed for available Zn in the whole soil (Figure 2(d)), though in soil solution from the overlimed condition, the opposite behavior of soil available fractions is observed. In this case, Zn availability could be explained due to the interaction of Zn with anions in soil, specifically with P, whose contents in the overlimed soil were reduced with the HA addition (data not shown). Since the increase in soil available P is negatively related to Zn soil availability and uptake (Marschner and Rengel 2012), P reduction may contribute to a Zn increase in solution of soil with pH 7.2 (Figure 2(d)).
Although the effects of soil applied HA to increase availability and uptake of micronutrients, mainly for Fe and Zn, have been reported in some studies (Chen et al. 2004; Garcia-Mina et al. 2004), the results found in this study showed that HA use was not able to increase Zn availability in both limed and overlimed soil conditions. Hartz and Bottoms (2010) also reported the absence of HA effects on soil nutrient availability, attributing this effect to the elevated levels of C from natural dissolved organic matter in the soil solution, which can perform some of the same functions of the exogenous applied HA. According to Olaetxea et al. (2017), positive effects of HS on crops are likely to be reached in soil nutrient solution with low C levels (0–50 mg L\(^{-1}\) dissolved C). In this work, high levels of C in soil solution were determined for the limed (~265 mg L\(^{-1}\)) and overlimed (~290 mg L\(^{-1}\)) soil samples; Figure 2(c)). Such high levels of dissolved C probably are attributed to soil disaggregation during soil sieve, which increases native organic matter decomposition rate (Balesdent et al. 2000). So, there was a great abundance of organic ligands provided by natural organic matter, and also by HA added to the soil, increasing the possibility of the formation of stable OMCs. In line with Garcia-Mina et al. (2004), pH and HS: metal stoichiometries are important factors regulating the availability of nutrients in soil due to the influence of both factors on solubility and stability constant of the organomineral complexes formed.

**Coffee seedlings growth and nutrition and their relation with HS application**

The highest production of DM of crops is frequently reported from soils with high and balanced nutrient contents (Khaled and Fawzy 2011; Lopes and Guilherme 2016). In line with that, data showed in Figure 6(a,b) illustrated the relationship between dry mass production and B and Zn contents in coffee plants of EI and EII. Besides, when HSs are used, high plant growth generally are reported and associated to improved soil fertility status and increased nutrient availability (Chen et al. 2004), or to the direct action of HS on plant physiology for both HS-treated soil or plant shoot (Canellas et al. 2015).

In EI, increase in soil pH to 7.2 caused a decrease in soil B and Zn availability, and therefore, the lowest coffee dry mass production and B and Zn accumulation (Figure 3(a,c); Figure 6(a)). Increase of soil B availability occurs in the limed soil treated with up to 38.5 mg kg\(^{-1}\) C-HA and in the overlimed soil with 25 mg kg\(^{-1}\) C-HA (Figure 1(b)). In overlimed soil, HA increased Zn in soil solution, at the concentration of 50 mg kg\(^{-1}\) C-HA (Figure 2(d)). However, the accumulated B and Zn in coffee SDM did not mirror the increased availability of these nutrients in the soil (Figure 3(e)). Probably, as discussed in item 4.1, organomineral complexes of B/Zn and HA were formed in both cases, increasing their soluble contents, but these complexes were not able to deliver micronutrients in time and amounts required by coffee seedlings. Another possibility is that HA application reduced availability of other nutrients (data not shown), and these impaired Zn and B adsorption and/or restrict plant physiological process rates. The general reduction of soil available Zn in both soil pH due to C-HA application is also a factor that may have reduced coffee seedlings growth (Figure 6(a)).

It should be also considered that the excessive addition of HS may block cell wall pores on the root surface, decreasing root hydraulic conductivity, which inhibits plant water uptake and shoot growth (Asli and Neumann 2010; Olaetxea et al. 2017). Based on the negative effects of excessive amounts HS in soil, use of HA concentrations up to 100 mg kg\(^{-1}\) in EI is also a possible explanation for the limited coffee growth.

In EI, since several reactions with soil and organic matter could reduce Zn and B availability when fertilization is done in soil, M\(_1\) application mode caused the lowest coffee seedlings dry mass, and B and Zn contents in shoot (Figure 5). B and Zn foliar application (M\(_1\) and M\(_2\)) resulted in the highest coffee DM, increased B and Zn content in shoot, mainly for sprays with the highest B and Zn concentration (M\(_2\); Figure 5). Therefore, the foliar micronutrient application mode is a suitable strategy to improve the agronomic value of FA in improving coffee seedlings growth and nutritional status.

Regarding FA effect on coffee plants, there was an optimum concentration to promote leaf dry mass and B content according to M\(_1\) and M\(_2\) application modes (Figure 5). Probably, up to the
optimum concentration, the FA had a stimulatory physiological effect on leaf membrane permeability, increasing the micronutrient uptake (Kaya et al. 2005; Khaled and Fawy 2011). Zancani et al. (2011) showed that FA increased ATPase activity in embryogenic cells, attributing this effect to its ability to act as an auxin-like molecule. ATPase acts creating an electrochemical driven force to cation uptake by H⁺ extrusion from the cell (Canellas et al. 2015). FA stimulation of specific micronutrient membrane carriers (Nardi et al. 2002) or active transporters stimulated by H⁺-ATPase activity (Jindo et al. 2016) is also a possible explanation for the positive effect of FA on plants. After the best C-FA concentration, reduction in leaf DM and B absorption could possibly be explained by the decrease of biochemical or physiological process rates (Moradi et al. 2017) and increased levels of reactive oxygen species and lipid peroxidation (Calvo et al. 2014), due to the excessive addition of FA. The increase of organic ligands availability provided by the elevated FA concentration could also result in the formation of organomineral complexes of high stability constant (Boguta and Sokolowska 2016), reducing plant acquisition of micronutrients. In line with Brown and Shelp (1997), B distribution in plants indicates its restricted mobility; however, B can be found in the phloem in B-polyol complexes that may re-translocated to plant sink organs that do not transpire. In part, the potential flux of B added in leaf to other plant organs like roots may explain suitable growth of coffee seedlings even for plants grown in soils with severe B deficiency.

FA application in EII did not improve Zn accumulated in coffee leaves, even when Zn was also sprayed on coffee leaf (M1 and M2; Figure 5(e)). Azcona et al. (2011) also found that HS promote significant increases in plant growth without change nutrient plant status. The authors attributed the positive changes in plant growth to the presence of hormones entrapped in the HS structure or to increased physiological process rates, photosynthesis efficiency along with alleviation of plant stresses (Anjun et al. 2011). However, considering that FA sprayed alone (M3), did not increase leaf DM, FA effects cited above, which are not related to leaf cell membrane permeability to micronutrients, could not be affirmed as action modes of FA on coffee seedlings.

It should be highlighted that even with a Zn decrease over C-FA concentrations in the M3, the content of Zn in the shoot was adequate for plentiful coffee growth (Martinez et al. 2013) and in higher content in coffee shoot than that found when Zn was supplied via soil (M3; Figure 4(f)). Such results show the suitability of choosing the right micronutrient application mode to improve coffee seedlings growth and its nutritional status.

Root-to-shoot ratio increased with C-HA addition in EI in the overlimed soil (Figure 3(d)). In the EII, on the contrary, a general decrease of root-to-shoot ratio was observed for M3 and the lowest value was observed at optimum C-FA concentration for M1 (Figure 5(d)). Root-to-shoot DM ratio mirrors the allocation of resources by the plants according to soil fertility status (Mokany et al. 2006). Increased values of root-to-shoot ratio are generally interpreted as a strategy of plants to explore more soil when growth conditions are not favorable to crops (Mokany et al. 2006). So, in EI, because HA reduced micronutrient availability, mainly Zn, less photoassimilates possibly were allocated for shoot and more for the roots. In EII, on the contrary, the reduced values of root-to-shoot ratio are indicative of favorable conditions to grow plants. At a first glance, a magnified plant root system, as in HA-treated plants could increase seedlings survival in coffee fields (Grossnickle 2004). However, root-enhanced development in coffee-HA-treated plants is reached at the expense of shoot growth, which reduced coffee biomass production as a whole.

It must be considered that HS acts majorly in the plant organ where it is applied (Nardi et al. 2002; Olaetxea et al. 2017). When added to soil or nutrient solution, several studies have reported that the increase of root growth and lateral root induction are the main responses related to the HS use (Canellas et al. 2002; Canellas and Olivares 2014). Thus, the HA soil application is more prone to stimulate root growth and FA foliar application, the shoot growth. In this way, the different local of HA-FA application in coffee seedlings organs could also be an explanation for the higher root-to-shoot ratio in EI and a lower in EII. Therefore, the results of this study showed that to truly evaluate the real agronomic value of HS, besides bioactivity, agronomic rate and crop degree of response to humic fractions, we must consider the mode (soil or leaf) of application, levels of C naturally dissolved in the soil solution of the cultivated soil and type of HS (HA or FA) supplied to coffee seedlings.
Conclusions

Soil application of HA did not improve coffee seedlings growth and B and Zn acquisition in both limed and overlimed soil conditions. Foliar application of FA combined with B and Zn was effective to improve the coffee leaves growth and B accumulation. In the high clay and rich dissolved carbon-Oxisol, use of FA, combined with B and Zn in foliar spray, is the most effective way to increase growth and acquisition of B and Zn by coffee seedlings.

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Disclosure statement

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