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Hydrochar from sugarcane industry by-products: assessment of its potential use as a soil conditioner by germination and growth of maize

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Abstract

Background: Hydrothermal carbonization (HTC) is a thermochemical process to convert biomass in carbon-rich materials (hydrochar). The use of sugarcane industry by-products in HTC has been evaluated, generating a hydrochar rich in nutrients, which could be used as a soil conditioner. We raised the hypothesis that the application of hydrochar in soil can improve its nutrient characteristics, bringing a better environment and favouring plant growth, expecting a development similar to that one observed in anthropogenic soils.

Results: Germination studies were performed expecting a species-dependent response, using maize and tomato seeds, whose development was assessed in two soluble fractions obtained from hydrochar aiming to evaluate different rhizosphere conditions. The results showed a better development of maize, especially in the aqueous soluble fraction, whose nutrient concentration was lower than that of the acid soluble fraction, as well as the organic composition. Maize growth in soils showed a better initial development in ultisol compared to oxisol, this being inferred by root:shoot biomass ratio and by scanning electron microscopy (SEM) images. However, the development of maize was better in anthropogenic soil compared to soils that received hydrochar.

Conclusion: The maize growth, compared with that carried out in anthropogenic soil, suggests that during the period evaluated the addition of hydrochar in soil did not have a negative effect upon maize development in its initial phase, and could have even favoured rooting in ultisol.

Keywords: Hydrothermal carbonization, Toxicity, Fertilizer, Anthropogenic soils, Plant growth

Background

Sugarcane appears as the third most cultivated crop in the world [1], and it is used as feedstock to obtain sugar and ethanol. Vinasse and sugarcane bagasse are the two main residues generated by ethanol production, and they are also considered by-products as they can be used for fertigation and energy co-generation, respectively [2]. However, an environment-friendly disposal for sugarcane industry by-products via hydrothermal carbonization has been proposed [3, 4]. This process generates a solid product (hydrochar), whose composition suggests that it can be applied as soil conditioner [3–5]. The characteristics of hydrochar may vary depending on the composition of the reaction medium. Some additives can therefore be

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added to the reaction medium to modify the solid product composition bearing in mind the final application, for example, as soil conditioner [5]. In addition, experiments in columns filled with soil showed that the hydrochar from sugarcane industry by-products could improve soil fertility by leaching nutrients and carbon to the soil [6], making them available for plant growth.

The high fertility of anthropogenic soils called *Terra Preta de Índio* (TPI), known in English as Amazonian Dark Earth, and *Terra Mulata*, rich in carbon and nutrients, is well known. These soils are located around the Amazon basin and are related to ancient occupation [7–9]. The high fertility has attracted the attention of researchers who showed that the carbon particles of these soils have a very aromatic core and a highly functionalized shell [10–12]. This composition is similar to that found in carbonaceous materials obtained from thermochemical processes such as pyrolysis and hydrothermal carbonization. These similarities have guided some studies that have tried to reproduce the anthropogenic soils' high fertility through the addition of carbonaceous materials, especially biochar (char obtained from biomass pyrolysis [13–16]), to soils [9, 17], as a way to improve soil fertility and to sequester carbon. One must also bear in mind that the presence of carbonaceous materials in soils might change its characteristics, such as nutritional content and aggregation [18–20].

The organic matter present in hydrochars is formed through several chemical reactions, thus forming very heterogeneous material [4, 5]. As they show wide diversity of composition, the characterization of the soluble fraction of hydrochars is also of great importance for understanding their role in the soil, as it is formed from a mixture of soluble organic substances, and is therefore the most mobile fraction of the material. As a result, the soluble fraction from hydrochar, combined with soil-dissolved organic matter, can jointly participate in processes such as nutrient cycling, transport of pollutants, and flow of CO_2 between the soil and the atmosphere, thereby having an influence upon biogeochemical processes in terrestrial environments [21, 22].

Different kinds of hydrochar have been evaluated as soil conditioners due to some similarities with biochar, such as high carbon content [23–28]. Studies showed that the response to application of carbonaceous materials, as hydrochar and biochar, to the soil depends not only on the biomass used in the thermochemical conversion process, but also on the reaction parameters applied [29–33]. Furthermore, these responses may be linked to hydrochar application rates [34], time after the application to the soil [35], and treatment carried out on hydrochar prior to this soil application [36, 37]. Positive results have been reported after 2 years from hydrochar soil application showing an improvement in biomass production [35], and also from pre-treated co-composted hydrochar used for plant growth, whatever the feedstock used to produce hydrochar. Best results were found for hydrochar treated with maize silage digestate and anaerobic fermentation, when compared to untreated hydrochar [38]. Moreover, the application response may be species-dependent, which means that the development of some crops can be favoured while others can be harmed [39].

Germination tests with process water from hydrothermal carbonization of sugarcane industry by-products were assessed by Fregolente et al. [40], reporting that maize seeds showed a better development in the initial phase due to the presence of process water [40]. These experiments represent a direct evaluation of what can be expected by the application of hydrochar from sugarcane industry by-products. However, the assessment of this hydrochar in soil still lacks. Thus, it is extremely important to conduct the evaluation of each material separately, by using different types of plants before a field application.

Therefore, the effects of hydrochar in soil may be assessed through plant development. We hypothesize that the presence of hydrochar in soils could release nutrients, thereby improving the soil's organic matter characteristics and promoting a better development of maize in the initial phase. Thus, to prevent the earlier effects of application of hydrochar, we first assessed the effects on maize and tomato germination. For this, experiments using extracts obtained from hydrochar in acidic and neutral conditions were performed, as the rhizosphere might show acid pH or neutral pH depending on the plant species cultivated and the soil type [41, 42]. Then, the comparison of maize development in soils (ultisols and oxisols) containing hydrochars was made with maize grown in *Terra Mulata* soils. We expected that hydrochars may improve soil characteristics leading to a maize development as good as that one observed in anthropogenic soils. The soil experiments may provide information for large-scale hydrochar application in the field, answering the questions about the feasibility, within the industrial sector, of the implementation of hydrothermal carbonization as a treatment process for by-products of the sugarcane industry [43].

Methods

Hydrothermal carbonization

The process of hydrothermal carbonization using vinasse and sugar cane bagasse (project registrated in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen), No. A0018C2), was assessed by Melo et al. [4] and the hydrochar produced was also characterized. The hydrochar

used in this work was obtained as described by these authors. Briefly, the hydrochar produced was made by application of vinasse and sugarcane bagasse at a 20:1 liquid-to-solid ratio (v/w). The reaction temperature was 230 °C and the reaction time was 13 h, using phosphoric acid 4% (v/v) [4], in a stainless steel handmade reactor. After the reaction time was completed, the reactor was immersed in an ice bath and the products separated by vacuum filtration.

The liquid phase (process water) was stored and the hydrochar was exhaustively washed with distilled water. This step is taking as a post-treatment for hydrochar, and it was applied in this work once better results for plant cultivation are reported when washed hydrochar is used instead of fresh hydrochar [32, 37]. After that, the hydrochar was oven dried at 50 °C until constant weight.

Soluble fraction extraction from hydrochar

The soluble fraction from hydrochar was obtained using two extractants, deionized water (AQ) [44] and a 0.1 mol L^{-1} solution of HCl (AC) [45]. For extractions, the ratio used was 1:15 of hydrochar (g) and extractors (mL). The mixture was mixed for 12 h, and then the aqueous phase was separated by centrifugation (3500 rpm for 15 min). The aqueous phase was then filtered twice, applying filter paper [45].

Characterization of soluble fractions

The total organic carbon concentration (TOC) of both soluble fractions from hydrochar was determined using a total organic carbon analyser (TOC-VCSN, Shimadzu). Fluorescence measurements were made using a spectrofluorometer (Cary Eclipse, Varian) in EEM mode. The spectra were acquired in the scan ranges of 300–600 nm for emission and 250–500 nm for excitation, with both slits fixed at 5 nm. The scan speed was set at 2400 nm min^{-1}, and the detector voltage was 700 V. For measuring the fluorescence, the concentration of each solution was adjusted to reach a 10 mg L^{-1} of TOC. This procedure was necessary to avoid inner filters on the fluorescence analysis [46].

The concentrations of Al, Ca, Mg, K, Cu, Fe, Mn and Na in the soluble fractions at carbon concentrations of 10, 50 and 100 mg C L^{-1} for both extractants were obtained by atomic absorption spectrometry by flame atomization (FAAS) (AA240FS, Varian), with Na and K being determined in the emission mode. Before FAAS measurements, the samples were submitted to acid digestion, following the 3010A EPA method [47].

Germination experiments

The germination experiments were performed using Petri dishes and agar as the medium for growth. Commercial seeds of maize (*Zea mays*) (Seminis, 85% germination) and tomato (*Solanum lycopersicum*) (Sakata, 95% germination) were used in the tests. The experiments were carried out as described by Fregolente et al. [40]. Hydrochar soluble fraction dilutions were obtained based on carbon concentration. Solutions with concentrations of 10, 50 and 100 mg C L^{-1} (represented by D0, D1 and D2, respectively). These were then pH-corrected to around 5.50 and used to prepare the agar medium [40, 48, 49].

Ten seeds were used for each Petri dish (15 cm diameter), with five replicates for each concentration. The Petri dishes were randomly placed in a germination B.O.D. chamber (MA 403, Marconi), maintained at 25 °C for 7 days, with a lighting schedule of 16 h light and 8 h darkness. The experiments were monitored daily, and the number of germinated seeds was recorded every day. The seeds were considered germinated when a length of 0.5 mm of shoot or root emerged from the seed. Finally, after 7 days, the total number of germinated seeds was recorded and the Germination Index (GI) was then calculated as described by Fregolente et al. [40]. The root and shoot lengths were measured using ImageJ software (version 1.51i).

Maize growth under different hydrochar application rates

The soils used in the experiments were: an ultisol collected near the city of Quatá, in São Paulo state (Brazil), and an oxisol from the region around Maringá, in Paraná state (Brazil), from a depth of 0–20 cm. In addition, a soil from Amazon Forest region called *Terra Mulata*, collected near Itacoatiara in the Brazilian state of Amazonas, was also used [the collection was authorized by the Chico Mendes Institute for Biodiversity Conservation, SISBio No. 50042-2, and registered with the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) No. A0018C2]. The soils were sieved (2 mm) to remove roots, plant remains and soil aggregates; then they were dried at room temperature before plant experiments. The information of soil particle distribution (texture) of these soils is available in Additional file 1: Table S2.

Hydrochar was applied at a rate of 0, 10 and 20 t ha^{-1} for oxisol and ultisol. For this purpose, 2.5 kg of soil were put in a plastic bag with the hydrochar at the appropriate rate and thoroughly mixed. Next, 500 g of the mixture were transferred to a plastic pot. For each concentration five replicates were made, these being kept for 90 days at field-capacity incubation, in a climactic chamber (TE-4002, Tecnal), at 26 °C, 50% humidity, in a photoperiod of 16 h light and 8 h darkness.

After the incubation period, 5 maize seeds (*Zea mays*) (Seminis, 85% of germination) were sown (approximately 2.5–3 cm depth). Eight days after emergence, thinning

was performed and just one seedling was allowed to grow for each pot. On the thinning day, each pot received an initial dose of fertilizer, with 70 kg ha^{-1} of P_2O_5, 70 kg ha^{-1} of K_2O and 70 kg ha^{-1} of N. The second fertilizer dose with 34 kg ha^{-1} of K_2O and 50 kg ha^{-1} of N was given 20 days after planting, respecting the NPK nutrients as required by the crop. The commercial fertilizer was added to all pots to ensure that the culture had sufficient nutrients as required to grow. This would ensure that any variation observed during the experiments was not due to a lack of nutrients.

The experiment lasted 40 days, after which the crop was harvested, and the root and shoot were separated. Plant tissues were washed with distilled water. Shoot and root dry matter were obtained by weight loss at 105 °C for 24 h. Samples from root, sheath and leaves were collected to be analysed by scanning electron microscopy.

Scanning electron microscopy (SEM)

The morphological changes in root, leaves and sheath were evaluated by scanning electron microscopy (SEM). The samples were fixed in a solution of 2.5% glutaraldehyde and 4% formaldehyde in a phosphate buffer of 0.1 mol L^{-1} at pH 7. The samples were then dehydrated in acetone, following an increasing series (30, 50, 70, 90, and 100%), and dried with hexamethyldisilane. Finally, the samples were fixed in stubs with carbon adhesive tape, sputter coated with 20-nm gold (Quorum QT150ES). The images were obtained using a scanning electron microscope (Quanta FEG 450—FEI) at a voltage of 20 kV.

Statistical analysis

The normality of the data was evaluated using *normal probability plots of residuals*, and the comparison between groups was performed using a general linear model (GLM). Tukey's post hoc test was performed when results were considered statistically significant, for a p value < 0.05. For statistical analysis, the Statistica software (version 8.0) was used.

Results and discussion

Soluble fractions characterization

The evaluation of hydrochar soluble fractions showed that the total organic carbon concentration (TOC) was higher in the acid (AC) fraction (313.6 ± 40.6 mg C L^{-1}) than in the aqueous (AQ) fraction (198.2 ± 19.9 mg C L^{-1}). Among non-volatile, semi-volatile and volatile organic compounds present in hydrochar from sugarcane industry by-products, as evaluated by da Silva [50] and Laranja et al. [51], carboxylic acids and their derivatives stand out, as also do phenols and benzene derivatives. Most of these present a long aliphatic chain, or show 2–3 condensed aromatic rings, which give them apolar characteristics [4, 50, 51]. So, the different pHs of the extracting solutions probably favoured the extraction of some compounds with functional groups containing oxygen as aliphatic esters, carboxyls and alcohols [52, 53], which would be present in higher concentrations in the AC fraction compared to the AQ fraction. As highlighted by da Silva [50] and Laranja et al. [51] the complexity of the identified compounds is high, mainly due to the composition of the biomass used in the hydrothermal process.

The characterization of the soluble fraction present in biochars has been explored, either qualitatively or quantitatively, through spectrophotometric techniques, such as EEM, but few studies regarding hydrochars have been reported [54, 55]. Figure 1a and b, respectively, illustrates the fluorescence spectra obtained in the EEM mode for the soluble AC and AQ fractions extracted from hydrochars. In general, both spectra contained peaks in several EEM regions, indicating the complex structure of fluorophores. The AQ fraction (Fig. 1a) showed a main and a secondary peak at [λEx 300 nm/λEm 430 nm] and [λEx 275 nm/λEm 300 nm], respectively. However, the AC fraction (Fig. 1b) showed two main peaks, one at [λEx 300 nm/λEm 430 nm] and another at [λEx 340 nm/λEm 410 nm], along with the secondary peak at [λEx 275 nm/λEm 300 nm].

The primary and main peaks were consistent with the results found for the soluble fraction extracted from biochars using water as an extraction medium, and it can be attributed to Peak C, which can be characterized by the presence of humic-like acid compounds with low molecular weight [56]. In addition, the secondary peak can attributed to Peak T, which may be assigned to soluble microbial by-product-like compounds, such as carbohydrates and proteins in both soluble fractions [57, 58]. The wavelength at which an organic molecule absorbs radiation is directly related to its molecular structure, thus providing a fingerprint of the sample [59]. As the attributions mentioned above were made to biochars extracts, and taking into account the existence of humic-like substances in hydrochar [60, 61], we hypothesized that the fluorescence peaks identified are also related to these humic-like structures, probably formed by aggregation of soluble organic compounds during hydrochar generation, and extracted mainly by use of AC solution.

Although the fluorescence peaks using different extractors were similar, the intensities were different. The maximum fluorescence intensity of an AC-soluble fraction was significantly higher than AQ, suggesting that at a low pH, more soluble organic compounds are extracted. Therefore, the decomposition of organic compounds present in the hydrochar that were not previously degraded by the HTC process, may have resulted in the greater release of acidic organic compounds in AC solution. As

fluorescence intensity is proportional to fluorophore concentration in diluted solutions, a greater presence of organic compounds was observed in the AC fraction, in line with the higher TOC value.

The concentration of nutrients in AC and AQ fractions are an indication of how the environment may change nutrient release. Considering the application of hydrochar in soils, their characteristics probably can also interfere with the amount of nutrients and carbon available for plant uptake [6]. However, the higher concentrations for almost all nutrients evaluated were observed in the AC fraction, excluding Cu and Na that showed similar concentrations for both AC and AQ fractions (Fig. 2). Higher concentrations for biochar acid soluble fraction of Mg, Ca, K, Fe and Na were also observed in the AC fraction by Sun et al. [45] in a similar study. This suggests that these nutrients may be more easily extracted in an acid solution.

In general, a bigger increase of nutrient concentrations accompanied by increasing of the carbon concentration (TOC) was observed in AC fractions, when compared to AQ fractions. Lou et al. [62] reported that aqueous extracts from maize and wheat biochars presented high concentrations in a descending order of K, Na, Ca and Mg, respectively. This is the exact opposite of what we found for AQ extract, which indicates that the nutrients and quantities released vary according to the carbon material characteristics, and, more specifically, vary according to the biomass used in the thermochemical process, which makes it difficult to establish a comparison between them.

Germination studies with hydrochar soluble fractions

Positive results regarding the use of process water from HTC of sugarcane industry by-products as liquid fertilizer were reported for maize and tomato seeds [40]. Now, we investigated the benefits that the hydrochar obtained from the carbonization process of the same biomass could provide to soil. The initial studies of the hydrochar application as soil conditioner were made with soluble fractions.

The results regarding hydrochar soluble fractions were similar to those obtained for the process water [40], with maize seeds growing better in the presence of soluble fractions than tomato seeds, with tomato showing more sensitivity to environmental variations. Further, we also observed an increase in the percentage of maize germination with the increasing of TOC concentration in AQ fraction (Fig. 3a, b), mainly for D1 and D2 samples; and a decrease in the percentage of maize germination with increased TOC concentration in AC solution. The germination index (Additional file 1: Figure S1) provided similar information, whereas in AQ the speed of maize germination seeds increased with TOC concentration, while the speed of tomato germination decreased. On the other hand, the speed of germination was not affected in AC, for both types of seeds.

Shoot and root development were better in AQ and AC fractions compared to the control group, especially for D1 and D2 concentrations (Fig. 3c, d). However, the results showed statistical differences for D2 AQ fraction, indicating the latter as the ideal concentration to promote maize growth (Fig. 3c). On the other hand, tomato seeds showed an increase in root and shoot in AQ fraction at the 10 mg C L^{-1} concentration (D0), and a decrease at D2 concentration. For the AC fraction, the results were similar to control for D0 and D1 concentrations, while development was negative at D2 concentration.

The differences in seed development in AQ- and AC-soluble fractions are probably due to the characteristics of extract solutions. The AC fraction released higher concentrations of nutrients present in hydrochar, e.g. Mg and Fe. In addition, the concentration of Al in all AC-soluble fraction dilutions was higher than the concentrations in AQ-soluble fraction dilutions. The concentration of Al

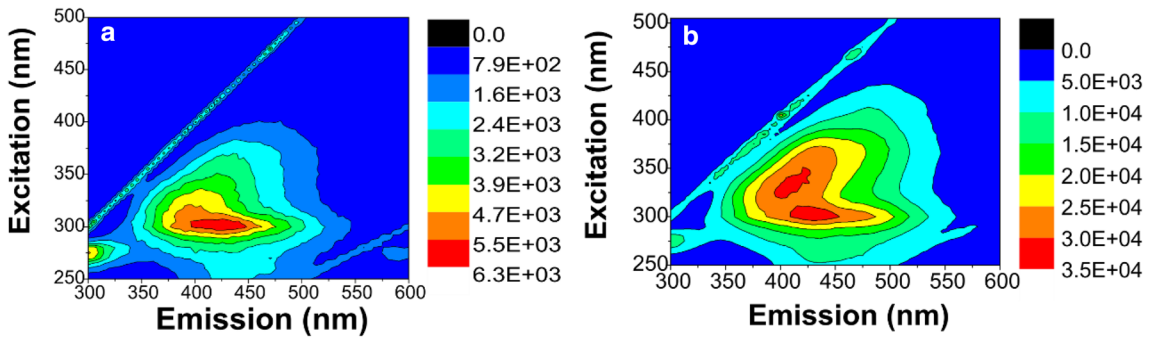

Fig. 1 EEM fluorescence spectra of **a** AQ- and **b** AC-soluble fractions from hydrochar

may be one of the limiting factors for seed development because, as reported by other authors, the presence of Al inhibited the development of roots and shoots of lettuce seeds at concentrations of 0.05–20 mg L^{-1} [63], and also of maize seeds at concentrations of 40–160 mg L^{-1} [64]. It is also reported that Fe at high concentrations has toxic potential (at concentrations higher than 10 mg L^{-1}) [65], meaning that Fe is another nutrient that might also have contributed to the low development of tomato roots and seedlings in the AC-soluble fraction treatments.

Sun et al. [45] evaluated the germination of maize seeds in acid extract (0.1 mol L^{-1} HCl) from biochar. Furthermore, they identified the inorganic concentrations in this extract and reproduced them to assess the germination and initial growth process under those conditions, to assess if the seeds' development was linked to inorganic or organic composition. The initial growth results showed no significant difference in seed development between the extracted solution and the artificially prepared solution [45]. However, besides the presence of some inorganic at high concentrations in AC fractions, another possible explanation for the better development of seeds in the AQ fractions may be linked to the organic compounds extracted.

The compositional characteristic of the organic compounds identified in the biochar extract by other authors [45] and in the hydrochar produced by the same process used in these germination tests [50] were similar, belonging to the class of compounds with similar H/C and O/C ratios, whose structures are characteristic of proteins, lipids, and lignin. Some studies have shown that compounds such as carboxylic acids, aldehydes and phenols may have an adverse effect on root and seedling growth [29, 65, 66]. However, in our study a positive statistically significant effect was identified especially when using AQ extract. EEM spectroscopy showed that the organic compounds present in the extracts may also be identified as humic-like substances, which may act like some hormones, responsible for promoting seedling growth [67, 68].

Therefore, despite the divergent results found for the extracts, it is possible to conclude that the characteristics of organic compounds extracted were probably different between the extracts, leading to different findings for AC and AQ seed development. While the acid solution probably extracted compounds responsible for inhibiting seedling growth in the case of the tomato, they did not have the same influence on maize. It was inferred that the aqueous solution extracted more bioactive compounds that stimulated root growth for both seeds assessed. Although the extracts showed the maximum fluorescence in the same region, the intensities were different. Hence, the rearrangement of the organic compounds apparently has different characteristics in the extracts. These observations, together with the increased concentrations in the solutions, led to the reported results.

Finally, for maize seeds, it is possible to infer that salt composition had an influence upon growth but was not a limiting factor, as results were positive for both extracts with the solution's carbon concentration increasing. However, it is worth mentioning that maize seeds development was favoured in the AQ fraction compared to the AC fraction. This allowed us to infer that this is a limiting factor as maize growth response is more linked to organic than salt composition. For the tomato, any AC fraction concentration showed positive results, which would suggest that, in the case of tomatoes, the development response is more sensitive regarding salt than organic composition.

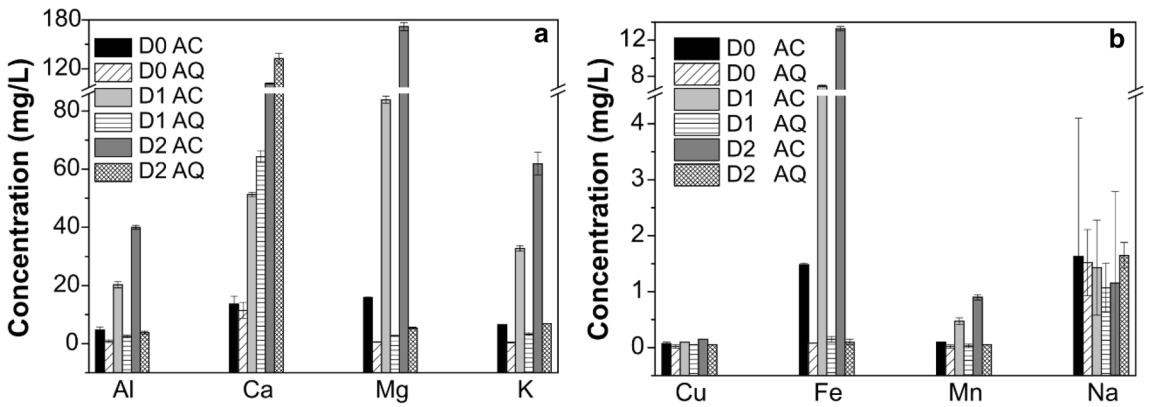

Fig. 2 Nutrient concentration in acid (AC) and aqueous (AQ) soluble fractions obtained from hydrochar to concentrations D0, D1 and D2

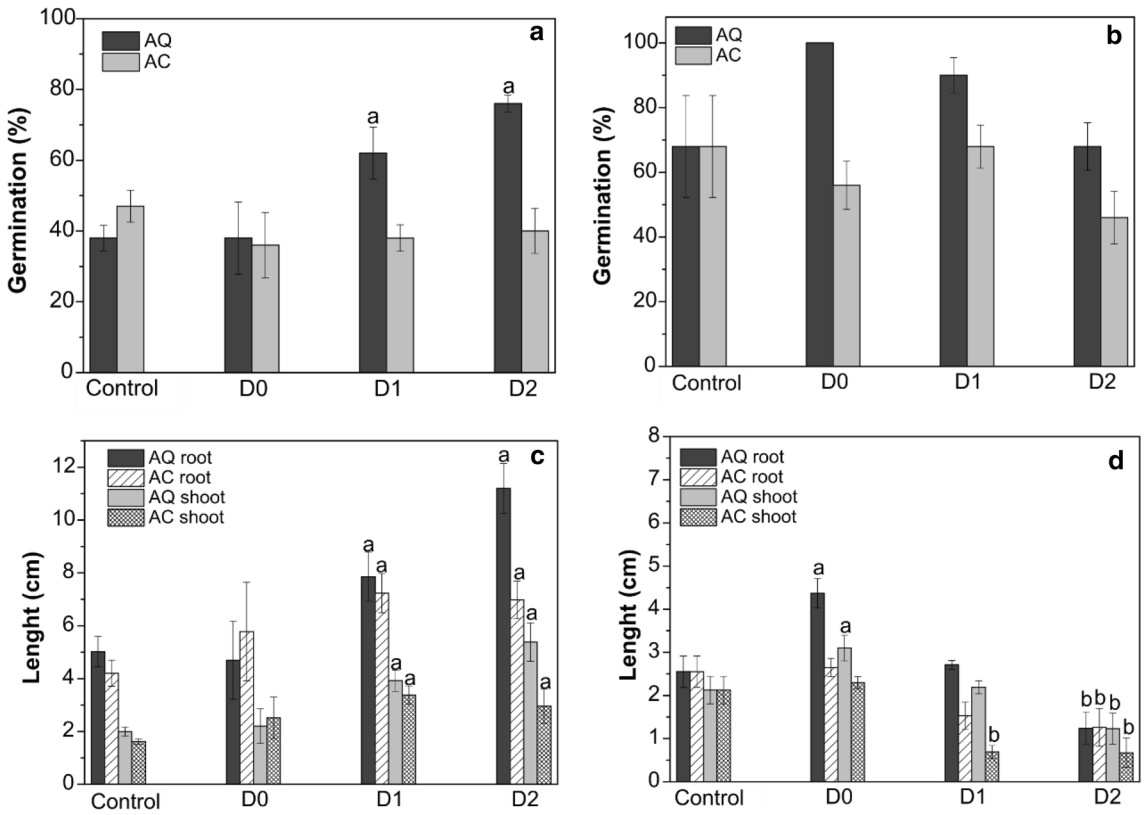

Fig. 3 Germination percentage of **a** maize and **b** tomato; shoot and root length of **c** maize and **d** tomato for different aqueous (AQ) and acid (AC) soluble fraction concentrations. [a]Indicates a positive statistically significant difference in relation to the control; [b]Indicates a negative statistical difference in relation to the control

Evaluation of maize growth in soil with hydrochar

The experiments on maize growth performed in pots using two kinds of soil were compared with the development in *Terra Mulata* soils, which are considered highly fertile. For this reason, *Terra Mulata* was used in this work as a positive control, as in this kind of soil one would expect better crop development. The development of maize in soil containing hydrochar at different rates was carried out at two moments: (i) 12 days after planting, and (ii) 60 days after planting. The first evaluation was carried out before thinning, at that moment all germinated seeds had their seedlings measured and number of leaves counted. Then, the seedling that showed the best stage of development was selected, and the others were removed from the pot.

On thinning day, evaluations of seedling length and number of leaves were recorded. The evaluations performed (Additional file 1: Table S1) showed a greater development in ultisol for 10 t ha^{-1} hydrochar application rate, and for oxisol in a rate of 20 t ha^{-1}. The initial growth observed for the 20 t ha^{-1} application rate in oxisol showed a positive statistical significance among all rates evaluated, including *Terra Mulata*. Differences among the number of leaves did not show significant differences among them or when compared to the control (soil without hydrochar).

Although a statistically significant difference was identified on thinning day, the dry matter analyses performed at the end of the experiment did not show any statistically significant differences between the application rates of 10 and 20 t ha^{-1} (Fig. 4). However, the shoot biomass production was bigger for all treatments with ultisols compared to oxisols. This difference was even verified when comparing with the control, which did not receive hydrochar, suggesting that the maize production may be preferable in low-clay soils.

Nevertheless, the development of maize in *Terra Mulata* was greater compared to all hydrochar treatments and kinds of soil evaluated, producing more shoots and root dry biomass, this being statistically proved. So, considering the dry biomass of root and shoot as an indicator of maize initial development and, therefore, for hydrochar as soil conditioner, the best

maize development was observed in *Terra Mulata* soils, followed by ultisol, mainly at the 10 t ha^{-1} hydrochar application rate.

Although significant positive results from hydrochar application have not been found, it was possible to observe an increase in dry root matter for 10 t ha^{-1} application rate, and mainly for shoot dry matter, compared to the control for ultisol. For oxisol, although no statistical difference was verified, a decrease in the shoot dry matter was observed for both application rates evaluated. Similar results were achieved by Melo et al. [69] for the first harvest of bean cultivation in soils with the addition of 4, 8, 16 and 32 t ha^{-1} of hydrochar. However, positive effects were observed in relation to dry mass for the second harvest, for which the application rate of 16 t ha^{-1} provided a 96% increase in biomass compared to soil without application of hydrochar [69]. In addition, they reported an improvement in soil conditions, in terms of nutrients, after the application of hydrochar.

The effects of hydrochar application in soils on maize growth were also investigated by scanning electron microscopy (Fig. 5 and Additional file 1: Figure S2). Although no statistically significant difference was observed between treatments and control, the microscale SEM images indicate that adverse effects of the application occurred mainly for oxisol. For the roots, the SEM images suggest structural alteration of the root cap tissue with an increase in the concentration of hydrochar in the soil (Fig. 5h, i), accompanied by changes in the characteristics of the root hair, showing flatness, with structural loss (Fig. 5c, j). For the leaves, an apparent decrease in the number of stomata was observed (Fig. 5k), which also makes one infer a water deficiency due to the growing conditions [70]. Also, an increase in the amount of wax on the sheath surface was observed, together with a change in the type of epicuticular wax, morphing into a granular form [71], showing a mechanism of the plant to prevent water loss (Fig. 5f, l).

These observations, accompanied by an increase of the root:shoot ratio (Table 1) with the increased hydrochar concentration in soil, led us to infer that the addition of hydrochar in oxisol hindered water absorption by the plants, since in conditions of low water availability the plants tend to allocate more biomass to the roots as a way to achieve greater efficiency to get this resource [72–74]. As the root:shoot parameter for *Terra Mulata* soil showed a value similar to that obtained in ultisol and oxisol control treatments, this adds weight to the idea that this difference is linked to hydrochar structure.

Hydrochars have hydrophobic characteristics due to the presence of aliphatic structures [3, 4, 75–77], even presenting a functionalized surface. This may indicate the presence of aliphatic groups on the surface of the coal. It also may be correlated to hydrophobicity of aliphatic parts identified in the humic-like substances present in hydrochar obtained from sugarcane and vinasse [68]. A structure with a more aliphatic characteristic would hinder the material's ability to interact with water, as observed to some biochars [78], interfering with hydraulic transport in soils, probably leading to a formation of aggregates, resulting in formation of preferential water paths observed mainly for the 20 t ha^{-1} treatment. So, this hydrophobicity could negatively influence plant development by making water availability difficult within the soil system. The particle size and material porosity may also contribute to intensify water repellence effects [79] as observed for the 20 t ha^{-1} application rate.

Apart from the factors linked to the structural characteristics of the material, according to the literature, the hydrophobic properties observed by the addition of hydrochar may also be due to certain fungi that can develop under the hydrochar surface, causing the water repellence effect observed [19]. Studies have shown that the lifetime of hydrochar in soils is shorter than that observed for biochar [80], explaining that the mineralization process for hydrochar is faster due to characteristics that facilitate the development and action of microorganisms [80, 81]. So, better results may be expected from application of hydrochar after longer interaction of hydrochar and soil, for instance harvest or in a subsequently cultivation, once the hydrochar degradation by mineralization process can release more nutrients present in the carbon material and also may reduce the water repellence observed [82–84].

For ultisol, the SEM images suggest an apparent increase in the amount of root hair in the region of

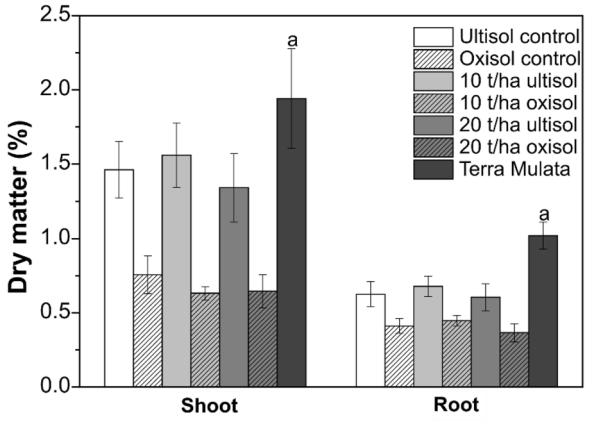

Fig. 4 Dry matter of maize shoots and roots collected at the end (60 days) of the growth experiment in oxisol and ultisol that received different rates of hydrochar, and in the experiments using *Terra Mulata* soils. [a]Indicates a positive statistically significant difference in relation to control and all treatments

maturation, showing the root hair be more elongated, mainly for the concentration of 20 t ha^{-1} (Fig. 5d). The increase in hair density can indicate not only a water sufficiency, but also a plant mechanism to optimize the acquisition of nutrients present in the soil in order to reach a larger exploitation area of soil resources through an increased the contact area [85]. These observations are in line with the soil nutrient content,

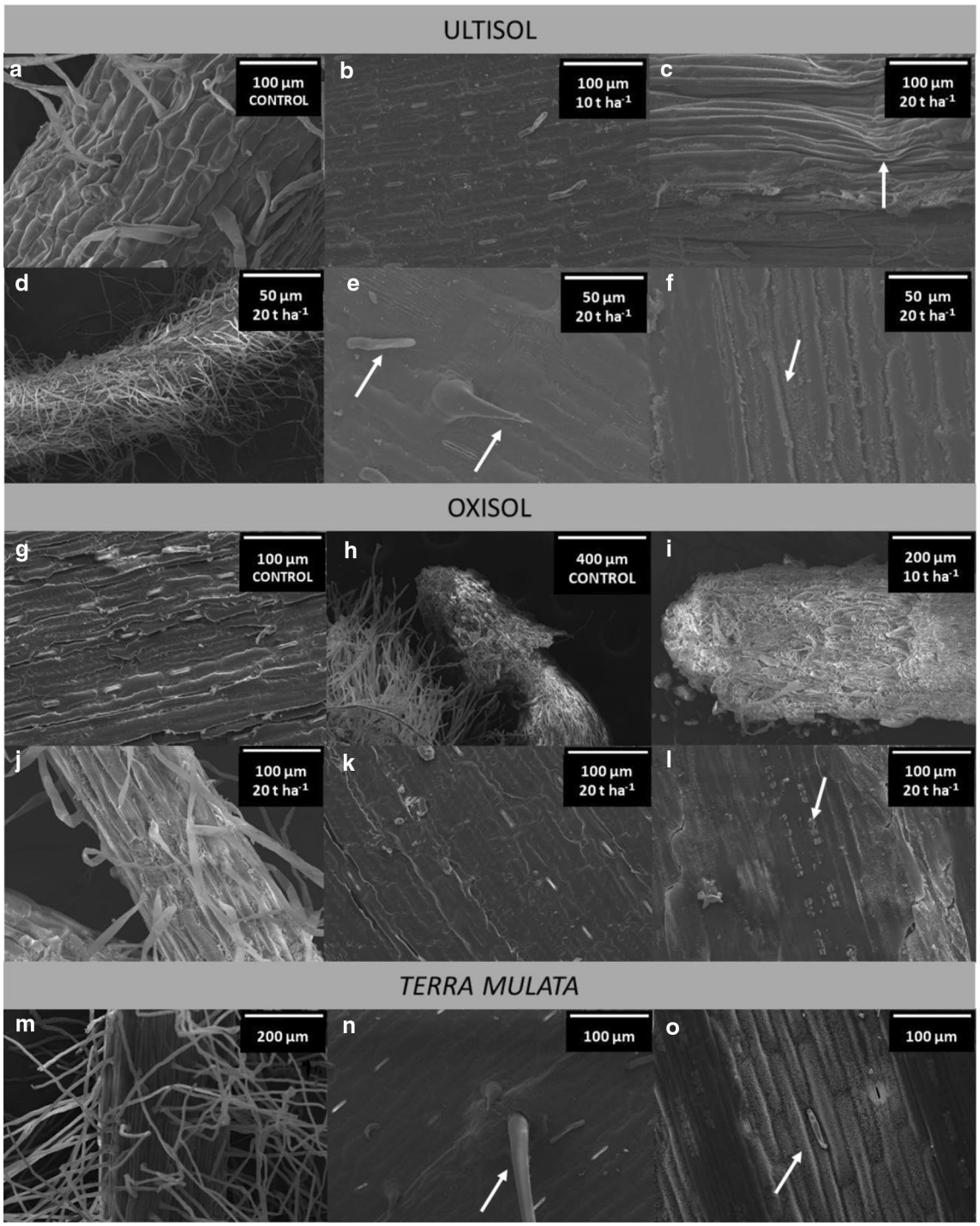

Fig. 5 SEM images of **a** root, **b** leaf, **c** root, **d** root, **e** leaf, **f** sheath of maize from ultisol; **g** leaf, **h** root, **i** root, **j** root, **k** leaf, **l** sheath of maize from oxisol; and **m** root, **n** leaf and **o** sheath of maize from *Terra Mulata* soil

as Bento et al. [6] showed that in sandy soils there is greater release of nutrients by hydrochar obtained with by-products of the sugar-energy industry.

In the leaf of the control group and the samples at 20 t ha^{-1}, we identified the presence of trichomes on the surface (Fig. 5e, n). The non-occurrence of these trichomes in the leaves at a concentration of 10 t ha^{-1} may suggest greater availability of water for this application rate, which did not require the development of mechanisms by the plant to reduce water loss [72, 86]. Furthermore, for the application rate of 20 t ha^{-1} it was possible to identify an increase in the amount of wax in the plants sheath (Fig. 5f, l). The root:shoot ratio for biomass for the experiments in ultisol did not change between treatments (Table 1), making it possible to infer that the development of plants was not influenced by hydrochar, inasmuch as the biomass allocation of both parts remained unchanged [72]. Comparing the SEM image results for ultisol with the *Terra Mulata* images (Fig. 5f, n, o), the same trichomes and the presence of wax were identified. Combining these results with the root:shoot ratio for biomass, we understand that such defence/adaptation mechanisms may be related to other factors, and not only linked to hydrochar.

In view of the results found in the literature, positive results from the hydrochar application may still be expected for subsequent crops, or even after a longer period of contact of hydrochar with the soil, since a longer incubation time of the material in the soil would help the mineralization process of both carbon and nutrients as present [80, 81].

Conclusion

It was understood that hydrochar soil application promotes different responses depending on soil characteristics and the application rate. The maize shoot and root dry biomass showed no significant change; however, sandy soil (ultisol) showed a better interaction with hydrochar, and this led to a development of shoot and root similar to the control. On the other hand, the hydrochar application at 20 t ha^{-1} in a clay soil (oxisol) indicates that water deficiency could be determinant to plant development. The SEM images of the plants suggested that the plants showed a better response when hydrochar was applied in ultisol, where evident signs of plant disorders were not verified.

Further, these results indicate that a longer incubation period of hydrochar in soil may improve soil conditions, especially in the case of sand soils, which may lead to a better biomass production among the crops. The use of *Terra Mulata* soil as a positive control helped to predict what performance can be expected from a good soil conditioner, and this in turn helped the process of evaluation, concluding that this hydrochar has potential as soil conditioner. Nevertheless, we suggest that experiments in the field, comprising all maize stages and with longer hydrochar–soil interaction be performed as a complement to these initial results.

Table 1. Determination of root:shoot dry biomass ratio for maize crop in soil treated with hydrochar and in *Terra Mulata*

	Root:shoot dry biomass ratio		
	Ultisol	**Oxisol**	***Terra Mulata***
Control	0.46 ± 0.10	0.45 ± 0.04	0.49 ± 0.08
10 t ha^{-1}	0.58 ± 0.07	0.72 ± 0.06	
20 t ha^{-1}	0.55 ± 0.06	0.63 ± 0.15	

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-021-00210-1.

Additional file 1: Figure S1. Germination Index (GI) maize and tomato seed germinated in acid (AC) and aqueous (AQ) hydrochar soluble fractions. **Figure S2**. SEM images of a) sheath, b) leaf, c) root and d) sheath of maize from ultisol; e) sheath, f) leaf and g) sheath of maize from oxisol. **Table S1.** Seedling length and number of leaves on thinning day, and number of leaves at the end of the maize growth experiment. **Table S2**. Soil particle characterization of Oxisol and Ultisol samples and their soil class.

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Authors' contributions

LGF conducted the maize growth experiments in soils and was the main contributor in the data processing and interpretation, also writing the manuscript. JVS performed the fluorescence measurements and data interpretation. FSM

performed germination experiments. TM and EM assisted the SEM analysis. ABM and OPF participated of conceptualization, formal analysis, methodology, writing - review and editing. MCB coordinated the research and was the supervisor of all phases of data analysis and interpretation, and reviewed the entire manuscript. All authors read and approved the final manuscript.

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