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Ecotoxicological assessment of biochar quality by using phytotoxicity bioassays

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Abstract

Soil amendment with biochar has been proposed as effective in improving agricultural land fertility and carbon sequestration, although the characterisation and certification of biochar quality are still crucial for agronomic acceptance. Described here are the effects of four biochars (conifer and poplar wood, grape marc, wheat straw) at increasing application rates (0.5, 1, 2, 5, 10, 20, 50% w/w) on both germination and root elongation of *Cucumis sativus* L., *Lepidium sativum* L. and

Sorghum saccharatum Moench. Biochars varied in chemical properties, depending on the type and quality of the initial feedstock batch; PAHs were high in conifer and wheat straw, while Cd and Cu exceeded maximum permitted values for amendments in poplar and grape marc, respectively. With our chars, electrical conductivity and Cu altered both germination and root elongation at ≥5% rate, together with Zn at ≥10% and elevated pH at ≥20%. Germination decreased only at very high rates of grape marc and wheat straw chars, whereas root length was affected already at 0.5% of conifer and poplar in cucumber and sorghum, with marked impairment in all chars at >5%. As a general interpretation, it is proposed here a robust root phytotoxicity logarithmic model in sorghum, based on biochar Zn content, which explains 66% of variability over the whole dosage range tested. We conclude that metal contamination is a crucial quality parameter for biochar safety, and that root elongation represents a stable test for assessing phytotoxicity at recommended amendment rates (<1-2%).

Key-words: Biochar, Feedstock quality, Germination bioassay, Metal contamination, Root phytotoxicity

1. Introduction

Biochar is a carbon-rich by-product resulting from waste plant material burned with little or no oxygen at very high temperatures, usually between 300 and 1,000 °C (Jeffery et al., 2011; Verheijen et al, 2010). In recent years, the importance of biochar for soil amendment has substantially increased, mainly as a response to increased global carbon emissions and deterioration of agricultural soil quality (Laird 2008; Lehmann, 2007). Due to its high porosity, specific surface area and carbon content, biochar can decrease nutrient losses and water leaching, and enhance soil cation exchange and water-retaining capacities (Chan et al., 2007; Lehman 2007). It can also adsorb and immobilise persistent organic and inorganic pollutants (Beesley et al., 2010; Hale at al., 2011; Oleszczuk et al., 2012; Fellet et al. 2014) and pesticides (Cao et al., 2011; Zheng et al., 2010) in

sediments and soils, decreasing the potentially noxious effects associated with their bioaccumulation through the food chain.

Despite these benefits, knowledge of soil-biochar interactions is still incomplete. Hazardous effects may derive from phytotoxic compounds, particularly heavy metals and polycyclic aromatic hydrocarbons (PAHs). Highly dangerous PAHs originate from degradation of lignin and cellulose during production (Freddo et al., 2012; Kuśmierz and Oleszczuk, 2014; Oleszczuk et al., 2013) and are adsorbed onto biochar surfaces (Sharma and Hajaligol, 2003). PAHs are of great environmental concern, due to their toxic, mutagenic and carcinogenic properties, and their presence may introduce unacceptable environmental, agronomic and human health risks when biochar is spread for soil amendment (Kuśmierz and Oleszczuk, 2014). Working parameters during burning of biomass (temperature, oxygen rate, supply feedstock rate, composition) can affect its chemical and physical properties (Spokas, 2010). Hence, it is essential to develop rapid and reliable procedures for biochar screening, to highlight the potentially negative effects on plant growth and human health before large-scale applications.

Within this framework, we compared the effects of four biochars obtained from a standardised gasification process and various feedstock batches, i.e., conifer and poplar wood, grape marc and wheat straw, on the germination and root elongation of three plant species routinely used in bioassay tests, i.e., *Cucumis sativus* L., *Lepidium sativum* L. and *Sorghum saccharatum* (L.) Moench. We aimed at: i) assessing the quality of different feedstocks, ii) identifying the best species as indicators of potential biochar toxicity, and iii) finding a relationship between biochar characteristics and phytotoxicity.

2. Materials and methods

2.1 Biochar production and characterisation

Biochars were obtained by gasification in a fixed-bed, down-draft, open-core, compact gasifier (AGT Company, Italy) at 1,200 °C constant temperature. Four feedstock batches were used: mixed conifer wood (CO), poplar wood (PO), grape marc (GM) and wheat straw (WS). The effects of biochars were compared with a reference commercial green-waste composted amendment (CA) obtained after 6-8-month maturation. pH was measured with a glass electrode on 10 g of pulverised biochar diluted in 25 mL deionised water, after 1 h shaking and subsequent stabilisation. Concentrations of trace elements were revealed in ~0.1-0.15 g DW homogenised samples after microwave-acid digestion (Milestone ETHOS 900, Bergamo, Italy). Samples were analysed by ICP-OES (SPECTRO CirOS Vision EOP, SPECTRO Analytical Instruments KG, Kleve, Germany). DTPA extraction was also performed following the Lindsay and Norwell protocol (1978). PAHs were quantified in 3-g samples treated with 50 mL of toluene (Sigma Aldrich, St. Louis, MO) for 3 h through Soxhlet equipment; the solvent was then evaporated and weighed. The residue was recovered with 1-2 mL toluene, adsorbed on a silica gel column and recovered by washing with 5 mL of toluene, concentrated to 0.1-0.5 mL, spiked with 200 µg of diphenyl (Sigma Aldrich) as internal standard, and injected (1 µL split mode 1/20 ratio) in a GC-MS analyser (Agilent Technologies, Inc., Wilmington, DE) equipped with a 30-m capillary column (0.25-mm i.d., 0.25 mcm f.t) connected with a 5-m silica pre-column (i.d. 0.53 mm). MS data were recorded at 70 eV scan mode (41-440 m/z).

2.2 Phytotoxicity bioassays

Three plant species were used for biochar tests, i.e., *Cucumis sativus* L. (cucumber), *Lepidium sativum* L. (watercress) and *Sorghum saccharatum* (L.) Moench (sorghum). Seeds from each species were obtained from plants not previously treated with fungicides. Seed vitality was

preliminarily assessed at 25±1 °C in deionised water, germination rates being generally >90% for all species. Seed germination and root elongation tests were performed according to OECD regulations (1984). The four biochars and CA were pulverised and mixed with a standard soil (SS) (70% quartz sand, 20% kaolinite, 10% finely-ground *Sphagnum* peat, pH 8.0±0.2) at 7 increasing w/w rates: 0.5, 1, 2, 5, 10, 20 and 50%, in comparison with SS alone, as untreated control. Tests were performed directly on soil matrices because the elutriates could not always reflect the true toxicity (Visioli et al., 2013; 2014). Four replicates per treatment were arranged by setting 15 g of substrate in 9-cm diameter disposable Petri dishes, covered with Whatman #1 filter paper and wetted with 5 mL of deionised water. Ten undamaged plump seeds were placed on the filter and the dishes were incubated at 25±2 °C in the dark for 72 h. Germination rate was evaluated as number of complete sprouts (≥ 1 mm long) of total number of seeds; shoot (sorghum only) and root lengths were also measured with a digital gauge.

2.3 Statistical analysis

To ascertain differences between biochars, CA and SS, "many-to-one" multiple comparisons were performed with Dunnett's test (Dunnett, 1955) as a follow-up to the one-way ANOVA procedure. Both endpoints of the phytotoxicity tests (germination and root elongation) were compared. When ANOVA revealed differences between CA and biochars, a multiple-comparison Tukey's HSD test was carried out. A log-logistic model from the Ritz and Streibig 'drc' R package (2005) was applied to fit the dose response of biochars and CA and to estimate the effective concentrations (Ec) responsible for reductions of 10, 30 or 50% in root length. In CO and PO biochars, the log-logistic model failed, and a linear interpolation was applied within the range 0-0.5% (which accounted for almost all variability). A multiple linear regression (stepwise), considering additive effects, was also used to verify the effects of different chemicals in biochars on the reduction in root length. To facilitate interpretation of the large dataset regarding biochar quality

and plant response, multivariate statistical analysis was applied at each tested biochar rate, to reduce the number of variables by PCA (principal component analysis) and to identify common data distribution patterns by cluster analysis. Before applying these, data were standardised by subtracting the mean and dividing by standard deviation within each variable.

Factorial discriminant analysis (MDA, Multigroup Discriminant Analysis, with Wilks' lambda and Pillai's trace tests) and PCA were applied to describe phytotoxicity and biochar quality based on germination rate and root length, and chemical features of biochars (i.e., pH, EC, total PAHs and metal rates: Cd, Co, Cr, Cu, Hg, Ni, Pb, Zn). Multivariate data normality was preliminarily verified by the Shapiro test. Multivariate cluster analysis was used to describe the characteristics of similarity among biochars and plant species. The data clustering algorithm was agglomerative (bottom-up) with distance optimisation and similarity (Pearson's correlation coefficient) as proximity method. The squared Euclidean distance and average link (UPGMA, Unweighted Pair Group Method using Arithmetic Average) were used as cluster distance and linkage method, respectively. In dendrograms, the maximum level of homogeneity within groups was calculated with the method of Calinski and Harabasz (1974). All statistical analyses were performed with R software (2013) and within MS Excel XLSTAT (Addinsoft, Paris, France).

3. Results

3.1 Chemical characteristics of biochars

Chemical characterisation of the four biochars revealed some differences in pH, metals and PAH contents (Table 1a,b). All biochars were alkaline, pH varying from 8.6 in poplar to 10.4 in grape marc. Only PO had a pH similar to that of SS and CA; the others were characterised by strong alkalinity, with pH generally >10 (Table 1a). GM also showed the highest electrical conductivity (EC >12 mS cm^{-1}), ~70× higher than that of PO and CA.

153 Metal concentrations were generally below the maximum admitted threshold recommended
154 by Italian legislation for amendments (Italian Legislative Decree 217/2006, in application of Reg.
155 CE n. 2003 13 October 2003). Metals above threshold were Cd in PO (+4%) and, in particular, Cu
156 in GM (+60%) (Table 1a).
157 Sixteen PAH species were revealed as the main pollutants, according to their potential mutagenic
158 properties (EPA, 2008). CO had the highest total PAH rate (>30 mg kg^{-1}), with considerably higher
159 concentrations of phenanthrene, anthracene and the carcinogenic benzo(b)fluoranthene compared
160 with the other feedstocks (Table 1b). GM had the lowest value (~5 mg kg^{-1}), the order being
161 CO>WS>PO>GM.
162
163 **3.2 Effects of biochars on seed germination and root elongation**
164
165 Seed germination of plant species was revealed at each tested biochar and CA rate (Fig. 1).
166 *Cucumis sativus* was not influenced at low rates, germination being significantly reduced with
167 respect to controls ($P<0.001$) at only >10% of GM and >50% of WS. *Lepidium sativum* showed an
168 increase in germination rate at all tested dilutions of CO and PO whereas, after initial enhancement,
169 GM again became severely phytotoxic at ≥5% and WS at ≥50% (-40% in germination). Similarly,
170 in *S. saccharatum* CO and PO did not inhibit germination, but was seriously affected above 10% of
171 GM and 50% of WS ($P<0.001$). GM was the most phytotoxic char, leading to complete inhibition
172 of germination at ≥10% in watercress and ≥20% in cucumber and sorghum. CA did not negatively
173 influence germination at any rate.
174 Root growth was severely affected by biochar amendment (Fig. 2). Root length in *C. sativus*
175 was significantly reduced at all biochar and tested rates ($P<0.001$). For this species, a marked fall in
176 root elongation (~80%) was already observed at 0.5% of CO and PO, followed by a stable response,
177 whereas phytotoxicity increased progressively with amendment rate in GM and WS. CA also
178 caused significant root inhibition compared with the untreated control ($P<0.001$) at any application

179 rate. Matching the complete inhibition of germination at ≥20% of GM, root elongation was also
180 impeded. *L. sativum* showed no inhibition in root length in CO and PO biochars, nor in CA, roots
181 even being stimulated at all tested rates ($P<0.001$); conversely, GM and WS, after initial stimulation
182 up to 1% and 2% of application, respectively, inhibited the root growth of this species; at 5% GM
183 and 10% WS, root lengths drastically fell, with significant differences with respect to controls
184 ($P<0.001$) (Fig. 2). Root and shoot lengths in *S. saccharatum* decreased gradually with increasing
185 application rates of CA, GM and WS ($P<0.001$), whereas marked impairment was immediately
186 observed at the lowest amendment rate (0.5%) for CO and PO ($P<0.001$) (Fig. 2).
187 Biochar application rates causing 10%, 30% and 50% reductions in root length for each species
188 were also estimated (Table SI1). Since estimates for PO and CO fell within the small application
189 range of 0-0.5%, in which the greatest variations occurred, they also provided the lowest values at
190 the three effective concentrations, i.e., ~0.06-0.09% (Ec_{10}), 0.12-0.18% (Ec_{30}) and 0.30-0.44%
191 (Ec_{50}), depending on species. Low Ec values were also found in GM in combination with sorghum.
192 The Ec_{50} of GM was quite stable across species (1.6-3.7%), but a larger variation was observed for
193 WS (7.1-14.7%). Matching the enhancement effect of all biochars within a 2% application rate (Fig.
194 2), *L. sativum* generally had a higher Ec compared with those of the other species (Table SI1).
195 ANOVA detected significant differences in root elongation between CA and biochars as average of
196 application rates. Pairwise comparisons confirmed great reductions in length with CO in *C. sativus*
197 and with GM in *L. sativum;* generalised growth impairment was observed in *S. saccharatum* (Table
198 SI2).
199 Stepwise forward linear multiple regression applied to the whole dataset identified only
200 electrical conductivity as a significant char parameter negatively related to root length, but the
201 model explained only slight variability ($R^2 = 0.15$). When analysis was broken down by species, the
202 Zn biochar rate appeared as the most important variable in both cucumber and sorghum, the
203 coefficients of determination being 0.36 and 0.39, respectively. A logarithm model in sorghum
204 turned out to provide the most suitable and significant fit ($R^2 = 0.66$) to describe root length (RL, %

of unamended control) over Zn concentration (mg kg^{-1}) corrected by biochar rate (BC%), as follows:

$$RL = 64.162 - 11.81*\ln(Zn*BC\%)$$

3.3 Principal component analysis and cluster analysis

PCA based on chemical characteristics of biochars and standardised seed germination rate and root length of plant species identified two dummy factors which explained 100% of variability. The first factor (F1) accounted for almost all variability, i.e., >89% in biochar classification and >77% in species classification, depending on amendment rate, so that only F1 is shown in Figs. 3 and 4.

At low amendment rates (0.5-1%) of biochar classification, F1 was supported (loadings > |0.5|) by electrical conductivity (EC), Ni and Cr, and seldom by PAH rate (Fig. 3). Although germination parameters only became significant at ≥5% amendment, germination was initially negatively correlated with EC and root length with Ni, Cr and PAHs (Fig. 3). Germination was negatively affected by EC and Cu at ≥5% amendment rate, together with Zn at ≥10% and elevated pH at ≥20%, as highlighted by the opposite direction of their vectors compared with those of germination rate and root length. In discriminant analysis, ellipsis overlaps and centroid positions generally highlighted two different groups, GM with high EC and Cu+Zn with maximum phytotoxicity, distinguished from the other biochars or commercial amendment. Only at 50% rate could a third group, represented by WS, be clearly plotted separately, with high pH and relatively high EC and PAH, capable of markedly reducing germination and root length.

With regard to species classification, root length significantly supported Factor 1 at all amendment rates, together with germination rate up to 2% of application. Both sorghum and cucumber were classified together as more sensitive species than watercress in highlighting phytotoxicity, with substantial indifference in species choice at 50% biochar rate only (Fig. 4).

At all amendment rates, the hierarchical ascendant classification of biochar-species interaction was a good descriptor of biochar type, regardless of choice of species (Fig. 5). According to the maximum level of similarity, the first group included poplar as the safest biochar source, with low EC, pH and Cu contamination, together with the commercial amendment; the second group included conifer and wheat straw. Grape marc was classified by itself in the third group, with critical salinity, pH and metal contamination.

4. Discussion

4.1 Feedstock and biochar quality

Biochar quality largely depends on the chemical and physical characteristics of the initial batch waste and gasification/pyrolysis parameters. Feedstock quality includes the rate of ash, together with plant nutrients and heavy metals, lignin, cellulose and hemicellulose rates, all having a substantial effect on chemical reactions during the semi-anoxic conditions of combustion and thus potentially leading to accumulation of undesirable and directly toxic compounds. This implies large variability in biochar composition and its impact on soil and plants, making difficult its certification.

Metal rates, together with EC and pH, are essential chemical characteristics for establishing biochar safety (Mukherjee and Lal, 2014; Lehmann and Joseph, 2009). In our experiment, biochar pH was rather variable but generally high, although we did establish its negative influence on plants at very high application rates (>50%) and in the literature the alkalinisation effect in the open is reported to be short-lasting (Lucchini et al., 2014). However, alkalinity is also related to high EC, which may be unsuitable for seed germination and initial root growth within wider ranges of application (5-50%). The characteristics of GM char, which was very rich in Cu as a result of common treatments

against fungal diseases in vineyards under both organic and conventional types of management, revealed the full effects of this metal. The illegal Cu rate is expected to have a direct phytotoxic effect on seedlings and to contribute to raising pH and EC. The good affinity of Cu to organic matter may mitigate its toxicity, but this probably did not occur in the crystalline structure of biochar, and the high Zn level also contributed towards GM phytotoxicity.

It is true that metal mobility is effectively reduced by high pH through stimulation of metal adsorption and precipitation (Beesley et al., 2011; Zhang et al., 2013), and we found moderate DTPA-extractable fractions of all metals in biochars, maximum bioavailability being for Cd in WS (~18% of total), followed by Cu in CO (~10%) and Zn in PO (~6%) (Table 1a). The considerable effects of heavy metals in germination occur at high biochar rates, and soluble metal fractions play a more important role in seedling growth than in completion of germination. Germination is certainly a delicate phase, but root elongation is more sensitive to adverse external conditions, a result already found in the case of high levels of soluble toxic metals in hydroponics (Vamerali et al., 2014).

Chemical characterisation of the four biochars revealed differences in PAH concentrations, conifer CO showing the highest rate (Table 1b). In soils, PAHs degrade slowly, and the high sorption capacity of biochars can extend their degree of environmental hazard over time (Kuśmierz and Oleszczuk, 2014; Quilliam et al., 2013), although our PCA results never indicated any important role of PAHs in seed germination and initial root elongation. Total PAHs includes several toxic compounds with generally low water solubility, and even the more soluble naphthalene and fluorene were very low or below detection limits. Root PAH concentrations are hardly altered by various types of biochars (Brennan et al., 2014), and in our case the absence of any direct root/substrate contact also excludes the possibility of demonstrating the influence of this compound class on seedling growth. The negative influence of wood-derived chars observed on root elongation is probably due to high Ni+Cr+Zn and Cd in CO and PO, respectively, some also exceeding maximum permissible levels for amendments. In markedly metal-contaminated

283 environments, biochar can also enhance root growth through metal immobilisation, indicating that
284 metal bioavailability is one of the main limiting factors in plant growth (Brennan et al., 2014).
285
286
287 **4.2 Species choice as indicator of biochar toxicity**
288
289 The main problem in biochar management of agricultural land is to identify and standardise
290 the chemical and physical indicators of quality (Lehmann and Joseph, 2009), in order to relate these
291 characteristics to the potential ecological and toxicological effects on soil-living organisms and crop
292 productivity (Lehmann et al., 2011).
293 Germination and root elongation are the most common tests in soil bioassays for checking
294 compound toxicity (Calvelo Pereira et al., 2010; Chigbo and Batty, 2013; Lin and Xing, 2007).
295 Several authors report that these tests are also effective in assessing biochar contamination
296 (Rogovska et al., 2012; Solaiman et al., 2012). As plant species may substantially differ in their
297 sensitivity to contaminants, our results show that seed germination was not greatly affected in all
298 species, whereas root elongation suffered to an extent which depended on choice of species (Figs. 1,
299 2). Although germination was reduced only at high biochar rate (e.g., 5-10% for GM), an earlier
300 response may be expected when metal contamination increases greatly, as in biochars derived from
301 wood treated with Cu-based preservatives, the Cu rate of which may be up to 60 times higher than
302 the rate we found (Lucchini et al., 2014). Instead, root length is considerably affected already at low
303 rates, as cell division/elongation in root tips is very sensitive to soil contaminants (Halušková et al.,
304 2010).
305 Feedstock quality plays an important role in biochar composition and phytotoxicity, but the
306 contrasting response among plant species to the same biochar was unexpected, e.g., the root length
307 of *C. sativus* and *S. saccharatum* was suddenly reduced by wood-based PO and CO biochars. Their
308 low effective concentration values (Ec_x) match recent finding on root elongation inhibition by

wood-based biochars (Jeffery et al., 2011). The behaviour of *L. sativum*, which shows root stimulation under wood-based biochars and CA, the latter also deriving from wood residues, and under low rates of GM and WS, is probably due to its higher tolerance to metal contamination. The *Brassicaceae* include several hyperaccumulator species, and cellular metal chelation is an accepted defence strategy (Anjum et al., 2012). This response suggests that bioassays should focus on the more stable behaviour of sorghum roots across the biochar types and CA, as evidenced by our Zn rate-based logarithm model. In *C. sativus,* Zn contamination was also the most suitable variable of this model (R^2 = 0.47), followed by pH (R^2 = 0.47), whereas the initial lag interval (up to 2-5% biochar rate) and the contrasting effects of chars in *L. sativum* caused the model to fail. For this species, electrical conductivity was the most constraining factor.

C. sativus is a sensitive and therefore key plant in phytotoxicity bioassays (Wang et al. 2001), although *S. saccharatum* seems a more informative species for biochar investigations. We were surprised to find that *L.sativum* was less sensitive, but this may partly depend on the relatively good quality of our chars and on the type of vegetal matrix, which affects contaminant mobility.

4. Conclusions

At present, there are no clear indicators for the agronomic acceptance of biochar, mainly due to uncertainty regarding its quality and difficulties in predicting interactions with plants and soil biota. Watercress, cucumber and sorghum can all reveal phytotoxicity by reducing their germination rate, albeit only at very high biochar rates. Higher sensitivity, compatible with recommended amendment rates (1-2%), can be easily retrieved from root elongation data. We demonstrate that metal contamination is the most critical constraint for plant growth, and increased metal loads in agricultural land can also exacerbate metal leaching and subsequent groundwater and food chain contamination. Although results on young seedlings must be confirmed in adult plants, these bioassays provide rapid information for char screening. Again, the lack of real long-term studies on

biochar effects in cultivated land, identifying contamination in the feedstock stream before gasification/pyrolysis remains a crucial step before large-scale applications of biochar become feasible.

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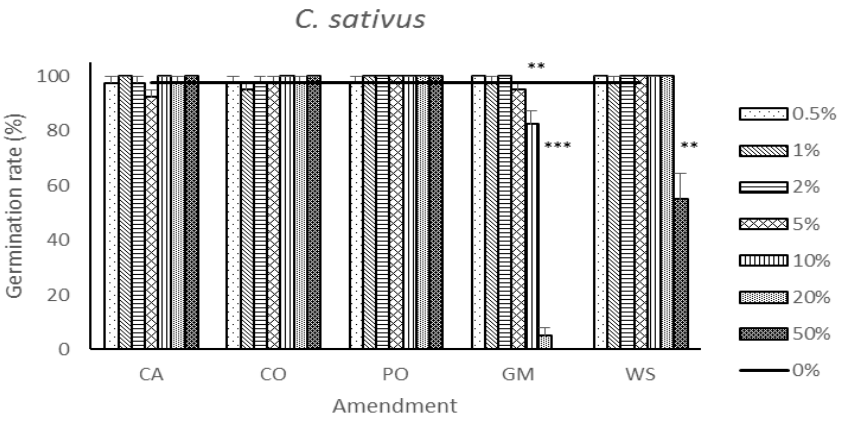

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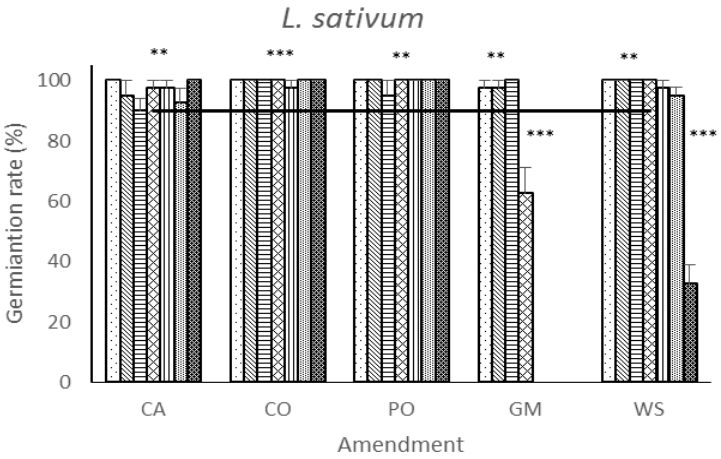

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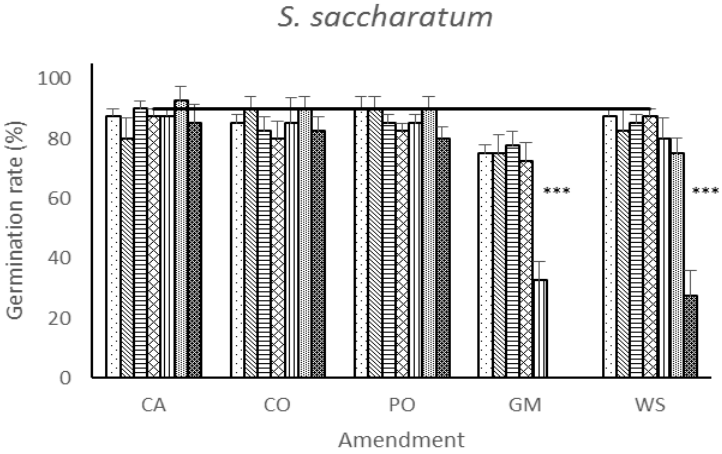

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456 **Figure 1**: Percentage germination (mean value ± SE, n = 4) of tested species at different biochar
457 rates, compared with commercial amendment (CA) and unamended control (horizontal bar).
458 Asterisks: significant differences between amendments and control (** = P<0.01; ***= P<0.001).

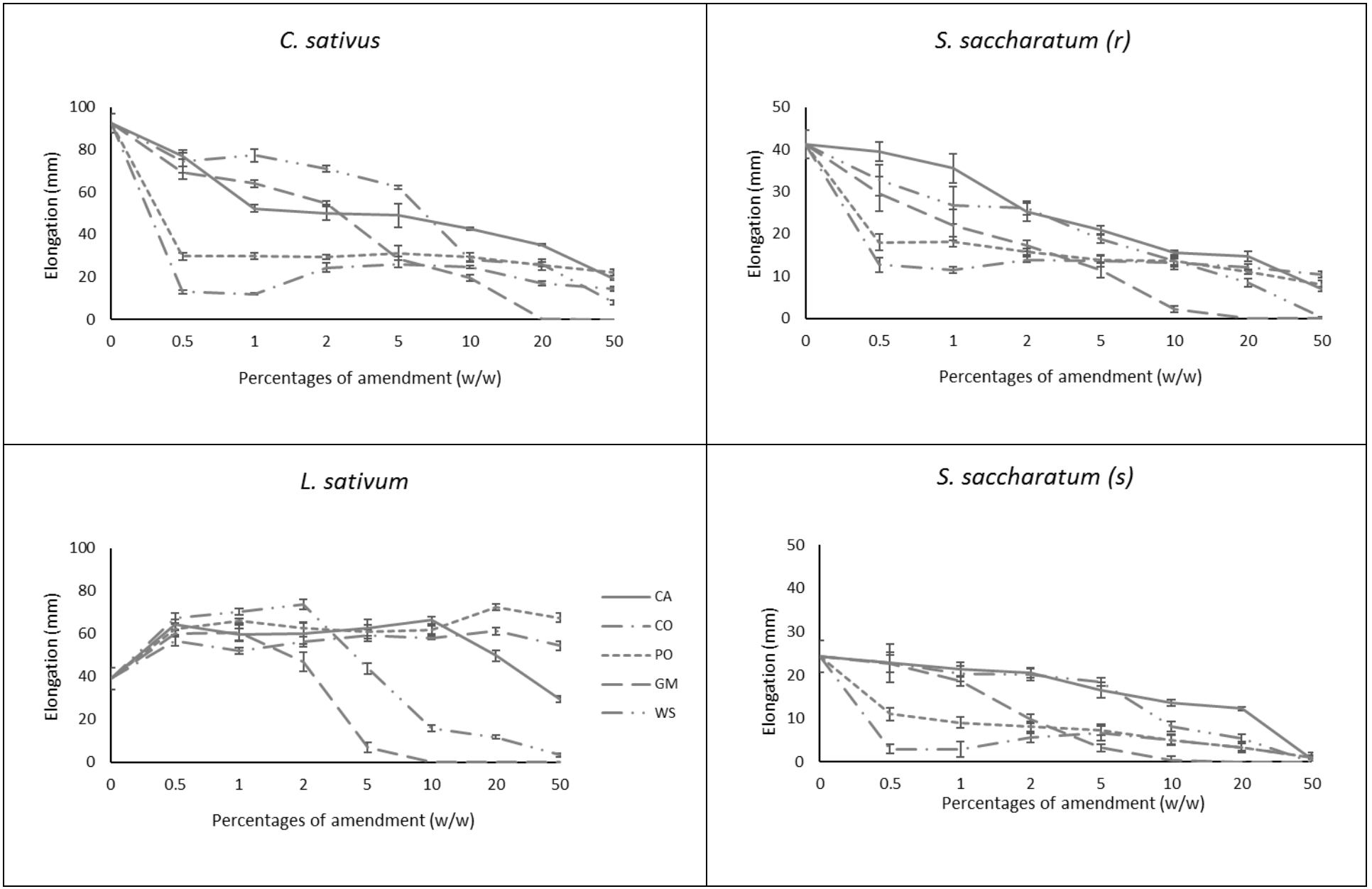
C. sativus
Elongation (mm)
100
80
60
40
20
0
0
0.5
1
2
5
10
20
50
Percentages of amendment (w/w)
S. saccharatum (r)
Elongation (mm)
50
40
30
20
10
0
0
0.5
1
2
5
10
20
50
Percentages of amendment (w/w)
L. sativum
Elongation (mm)
100
80
60
40
20
0
0
0.5
1
2
5
10
20
50
Percentages of amendment (w/w)
CA
CO
PO
GM
WS
S. saccharatum (s)
Elongation (mm)
50
40
30
20
10
0
0
0.5
1
2
5
10
20
50
Percentages of amendment (w/w)

Figure 2: Shoot (s) (only sorghum) and root (r) lengths (mean value ± SE, n = 4) after germination experiment in three species under increasing biochar rates, compared with commercial amendment (CA). Note different scale among graphs.

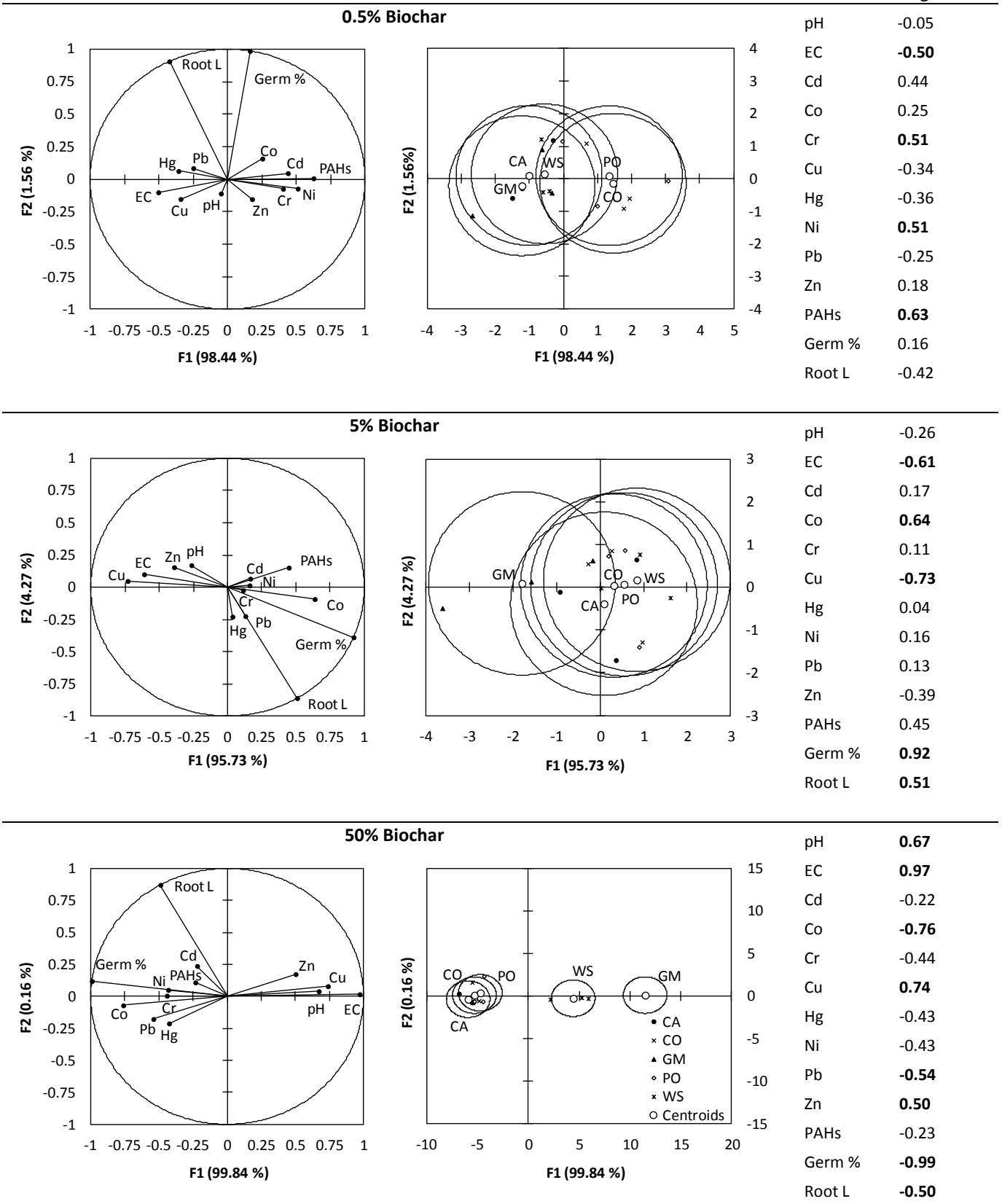

Figure 3: PCA with F1 loadings (highlighted values > |0.5|) and DA for biochar classification at 3 selected amendment rates, considering chemical characteristics of biochars, germination rate (Germ %) and root length (Root L).

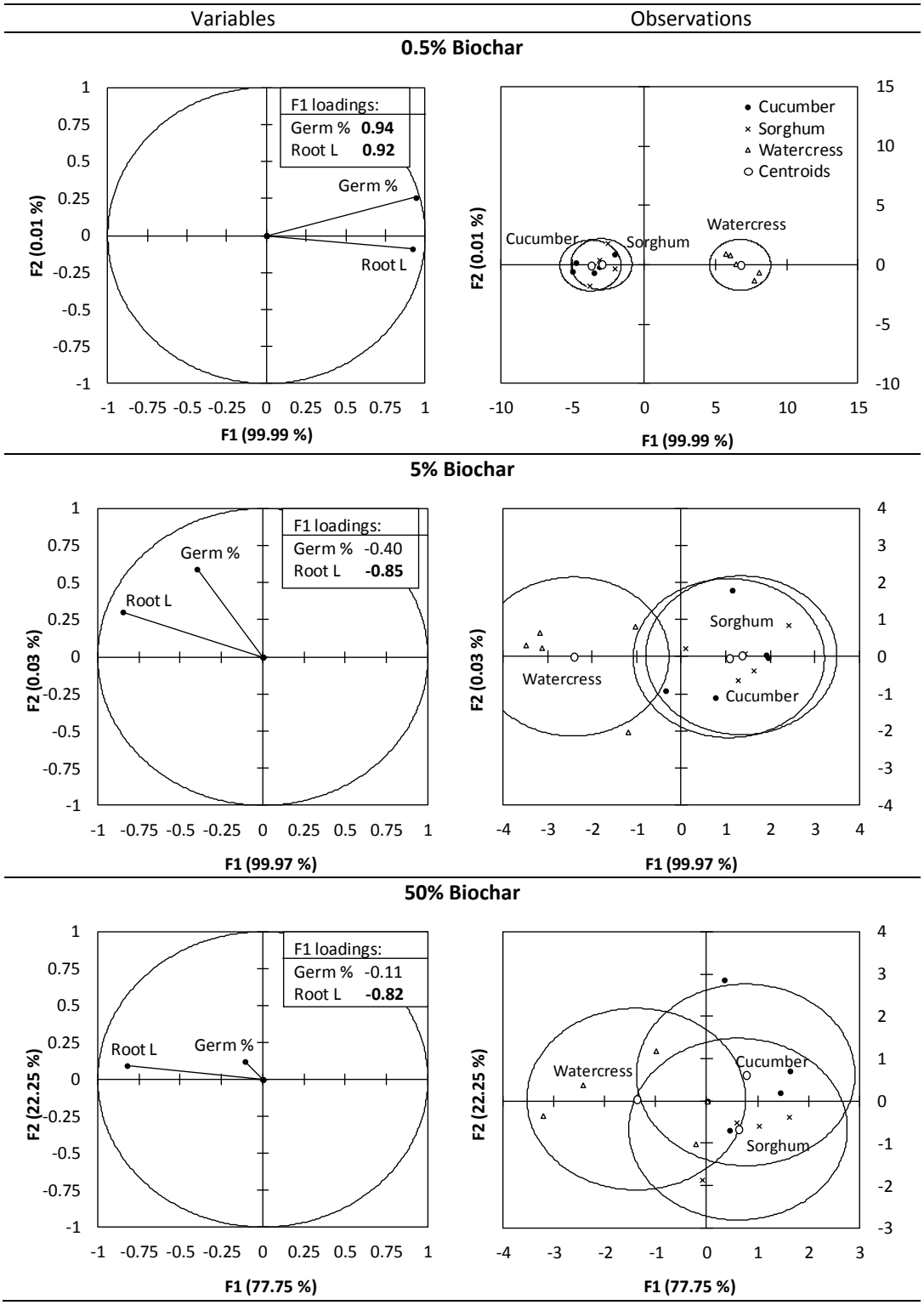

Figure 4: PCA and F1 loadings (highlighted values > |0.5|) for species classification at 3 selected amendment rates, considering germination rate (Germ %) and root length (Root L). Loadings of chemical characteristics of biochars: always nil.

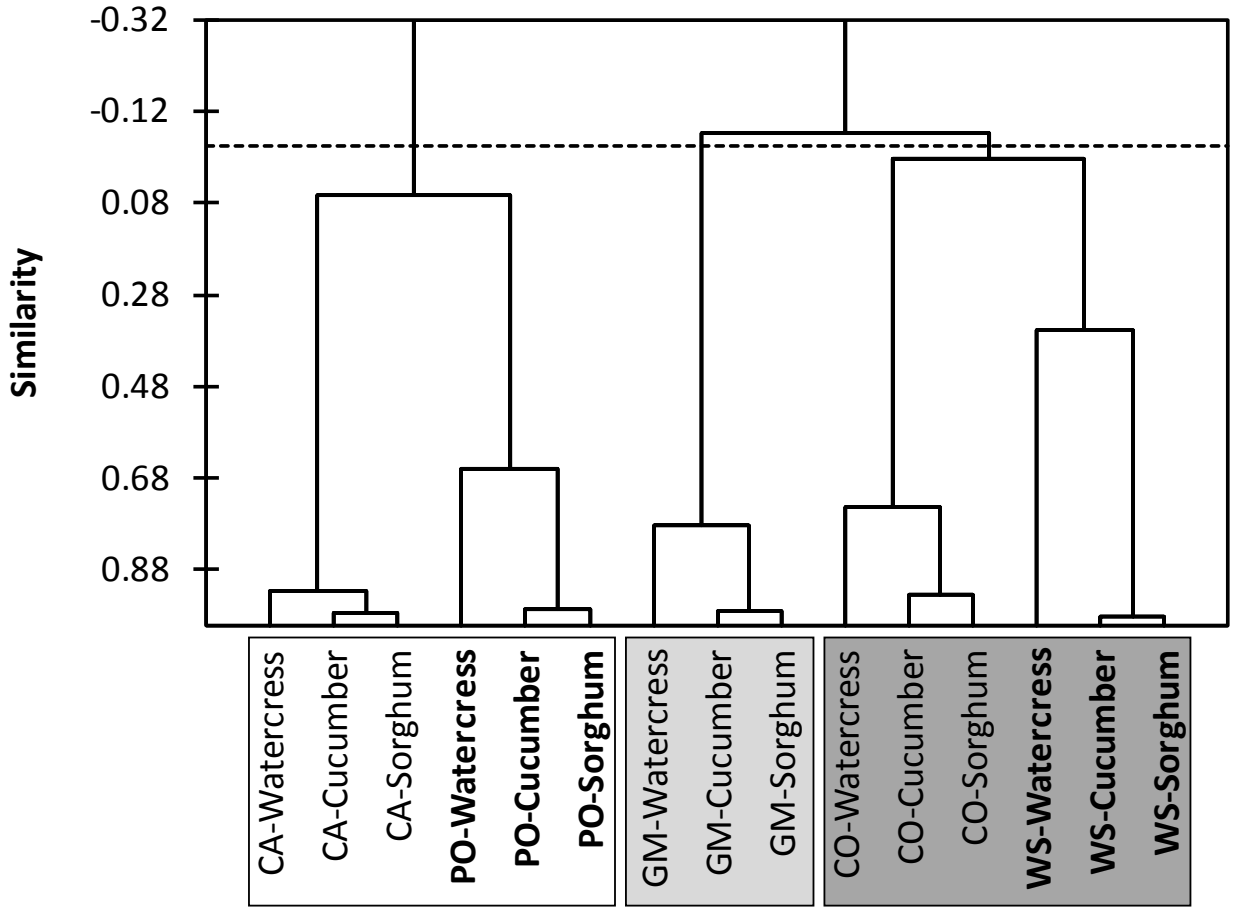

Figure 5: Cluster analysis of biochar/species combinations at 0.5% amendment rate, based on chemical characteristics of biochars and germination rate and root length of 3 species. Horizontal dashed line: maximum level of homogeneity within groups. Similar classification obtained at all amendment rates.

476 **Table 1a:** Main chemical proprieties of biochars in comparison with standard soil (SS) and
477 commercial amendment (CA)
478

	IGV	SS	CA	CO	PO	GM	WS
pH	-	8.0	8.2	10.2	8.6	10.4	10.1
EC (mS cm^{-1})	-	ND	0.15	0.62	0.17	12.2	4.79
Cd (mg kg^{-1} DW)	1.50	<0.001	0.29	0.34 (12.7)	**1.56** (14.3)	0.47 (8)	0.41 (17.8)
Co (mg kg^{-1} DW)	-	6.13	2.71	2.05 (<3.1)	2.74 (<3.1)	1.29 (<3.1)	2.43 (<3.1)
Cr total (mg kg^{-1} DW)	-	89.2	47.1	114 (1)	23.5 (<0.2)	30.3 (<0.2)	29.5 (<0.2)
Cu (mg kg^{-1} DW)	230	3.56	53.8	111 (10.1)	34.9 (3.3)	**369** (1.8)	26.5 (5.5)
Hg (mg kg^{-1} DW)	1.50	<0.002	<0.002	<0.001 (-)	<0.001 (-)	<0.001 (-)	<0.001 (-)
Ni (mg kg^{-1} DW)	100	53.0	22.0	85.4 (0.7)	18.9 (1.6)	16.2 (0.7)	19.4 (1.8)
Pb (mg kg^{-1} DW)	140	7.32	27.7	6.34 (3.6)	10.7 (4.8)	5.23 (<2)	6.92 (2)
Zn (mg kg^{-1} DW)	500	25.0	106	272 (4.7)	180 (6.2)	282 (2.2)	183 (4.1)

479
480 Highlighted values exceed Italian Guidelines Values (IGV) for total metal rate in amendments. In
481 brackets: % of metal bioavailability (DTPA-extraction).The analysis was performed in duplicate.
482 ND = not detected
483

484 **Table 1b:** Polycyclic aromatic hydrocarbons (PAHs) rates (mg kg^{-1}) in biochars.
485

PAHs	CO	PO	GM	WS
Naphtalene	ND	0.1	0.19	ND
Acenaphthylene	0.27	0.15	2.29	ND
Acenaphthene	0.05	ND	ND	ND
Fluorene	ND	ND	ND	0.19
Phenanthrene	5.29	2.08	ND	1.41
Anthracene	0.93	0.44	ND	0.23
Fluoranthene	3.12	1.28	0.13	4.91
Pyrene	3.80	2.14	0.37	4.95
Benz(a)anthracene	1.40	1.50	0.07	1.22
Chrysene	1.33	0.87	0.09	1.14
Benzo(b)fluoranthene	2.16	1.74	0.06	0.97
Benzo(k)fluoranthene	0.00	1.41	0.10	ND
Benzo(a)pyrene	1.20	1.62	0.10	0.45
Dibenz(a,h)anthracene	ND	ND	ND	ND
Benzo(ghi)perylene	0.88	1.72	0.14	0.27
Indeno(1,2,3-C,D)pyrene	0.63	0.61	0.26	0.09
Total of 16 PAH	21.06	15.66	3.81	15.84
Other PAHs (calculated without response factors)	11.60	5.25	1.24	9.73
TOTAL PAH	32.66	20.91	5.05	25.57

486
487 The analysis was performed in duplicate. ND = not detected
488

Table SI1: Effective concentration (%) for biochars at 10, 30 and 50% root length reduction, i.e., Ec_{10}, Ec_{30}, Ec_{50} in three plant species.
n.p. = non phytotoxic

Biochar	Plant species	Ec_{10}	Ec_{30}	Ec_{50}
CO	*C. sativus*	0.06	0.12	0.30
	L. sativum	n.p.	n.p.	n.p.
	S. saccharatum	0.07	0.14	0.36
PO	*C. sativus*	0.07	0.15	0.37
	L. sativum	n.p.	n.p.	n.p.
	S. saccharatum	0.09	0.18	0.44
GM	*C. sativus*	0.3	1.4	3.3
	L. sativum	2.4	3.1	3.7
	S. saccharatum	0.1	0.6	1.6
WS	*C. sativus*	0.9	3.3	7.1
	L. sativum	3.6	5.0	6.2
	S. saccharatum	0.2	2.8	14.7

Table SI 2: Effects of biochar type on root elongation (main effect, i.e., mean of various amendment rates) of plant species at end experiment in comparison with the commercial amendment CA (Tuckey's HSD test; $P \leq 0.05$). Letters: statistical significant differences among chars within same species.

Species	Biochar/Amendment	Root length (mm)
C. sativus	CA	46.4 a
	CO	19.1 c
	GM	33.7 b
	PO	33.9 b
	WS	49.7 a
S. saccharatum	CA	19.2 a
	CO	12.5 b
	GM	11.0 b
	PO	13.5 b
	WS	17.8 a
L. sativum	CA	56.1 a
	CO	56.8 a
	GM	24.9 c
	PO	64.9 a
	WS	40.9 b