Biochar
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ORIGINAL ARTICLE

Biochar-induced changes in metal mobility and uptake by perennial plants in a ferralsol of Brazil's Atlantic forest

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Abstract
Despite an abundance of short-term studies focusing on biochar's effects on annual plants, the long-term effects of biochar on perennial plants and the effects of the biochar on the mobility and speciation changes of metals/metalloids not limited to main plant nutrients in soils are poorly constrained. This study reports on the amelioration a sloped orthic ferralsol by biochar from *Tibouchina* wood and the resulting effects on perennial crops and microbiota, including a comprehensive analysis of metals/metalloids speciation changes. Fields were amended with biochar and urine-amended biochar (2 kg/m^2) and were planted with papaya, banana, and manioc. Soil and plant materials were analyzed using acid digestions, sequential extractions, and 16S rRNA gene sequencing. Biochar applications led to decreased soil acidity, shifted the cation exchange capacity from being Al-influenced to being Mg/K/Ca-dominated, and elevated the concentrations of Mg, K, Ca, Zn, and Ba in soils. The exchangeable/acid-soluble fraction of Ca, P, and S notably increased. The soil microbial biome became more species rich and diverse in the biochar-amended fields. Manioc benefited from biochar applications, demonstrating increased growth, which resulted in generally decreased concentrations of trace elements in most plant parts, however, with an increased total elemental uptake. Urine amendment contributed to higher concentrations of P, S, and K in soils, but did not further increase plant growth. Biochar was shown to be a promising soil amendment for agricultural use of orthic ferralsols of the Brazil's Atlantic forest region, but the accumulation of potentially harmful metals needs to be considered.

Keywords Biochar in agriculture · Agroforestry · Ferralsol management · Trace-metal speciation · Biogeochemistry · Plant metal uptake

1 Introduction

Biochar, or pyrolyzed biomass, can be used to beneficially alter soil properties, e.g., increase soil moisture, lower nutrient losses, increase soil pH in acidic soils, and provide a suitable substrate for microbial communities (Jeffery et al. 2011; Lehmann and Joseph 2015; O'Connor et al. 2018; Shaaban et al. 2018). Often, animal waste products or bio-solids are used as the biochar feedstock (see selected studies listed in Table 1). However, biochar itself can carry high concentrations of macro- and micronutrients that can leach into the soil over time. This is certainly the case for wood-derived biochars which have been shown to contain large fractions of exchangeable and acid-soluble metals (von Gunten et al. 2017). The availability of metals in wood-derived biochar, which are important for plant growth, makes such materials a promising candidate for soil amendment.

While biochar has been reported to strongly interact with trace metals, such as Ni, Zn, and U (e.g., Alam et al. 2018a; b) and can alter soil metals concentrations (Table 1), much uncertainty still exists about the relationship to observed improvements in plant growth. As a first

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Table 1 Selected effects of long-term soil amendments with biochar

Soil type	Period	Biochar feedstock	Plant type	Additives	Major findings	References
Xanthic Ferralsol (Brazil)	2 years	Secondary forest wood	Rice and sorghum	Compost and mineral fertilizer	Increased soil nutrient availability (including exchangeable metals) and higher crop yields	Steiner et al. (2007)
Slightly acidic soil (Indonesia)	2 years	Farmyard manure or manioc stems	Intercropped systems (manioc, peanuts, and maize)	N/A	Longer lasting but more moderate fertilizing effect compared to pure manure	Islami et al. (2011)
Gleysoil (Philippines)	4 years	Rice husk	Rice	N/A	Increased plant-available C, N, P, and K	Haefele et al. (2011)
Eutric Cambisol (United Kingdom)	3 years	Fodder maize	Grassland plants	Herbicide simazine	No significant effects on C and N; formation of a bacterially dominated decomposer community beneficial for soil fertility; reduction in K, Na, and Ca	Jones et al. (2012)
Cerrado soil (Dystric Plinthosol) in Brazil	2 years	Eucalyptus	Rice	Mineral fertilizer	Increased availability of Ca and P; decreased Al acidity in the top soil layers, but reduced effects in the second year	Petter et al. (2012)
Eutric Cambisol (United Kingdom)	9 months	Deciduous trees	Field bean, spring barley	N/A	No significant changes in the concentrations and speciation of heavy metals; slight increase in soil B, K, and Mg concentrations	Lucchini et al. (2014)

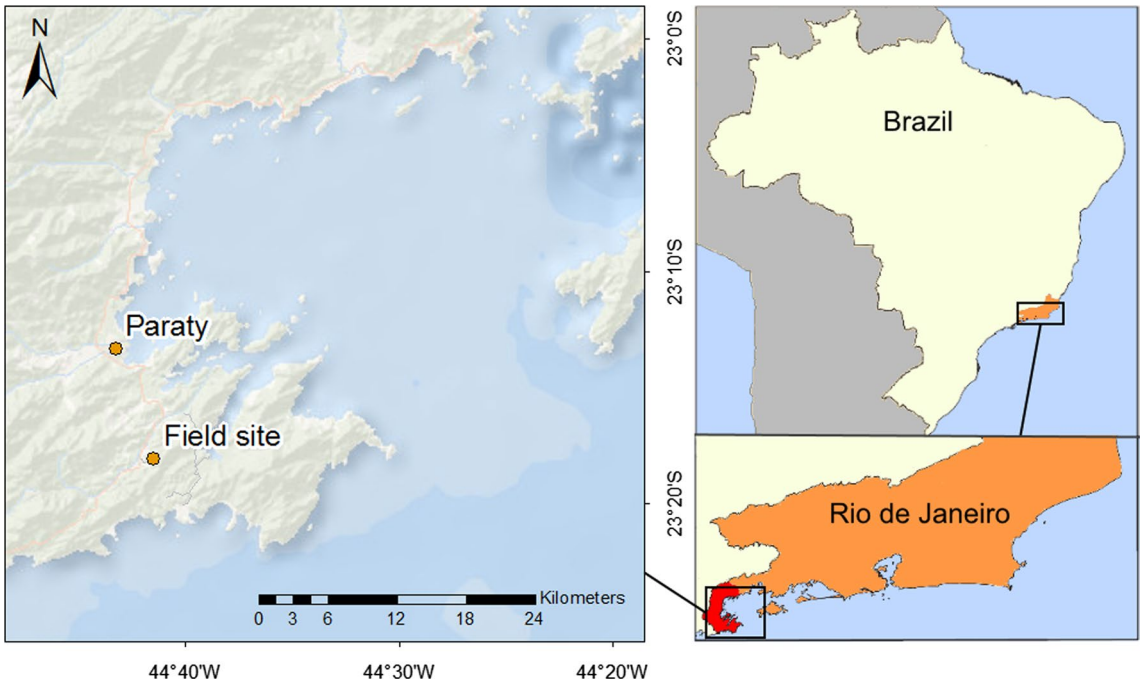

Fig. 1 Location of the field site

step, the metal distribution (speciation) in various soil fractions needs to be studied to assess potential changes in mobility and bioavailability of these metals in addition to concentration effects. Such analyses are more often performed when evaluating soil contaminant immobilization by biochar, such as Cd, Pb, or Ni (Bian et al. 2014; Liqiang et al. 2016; Shen et al. 2016; Novac et al. 2019), but are often neglected in agricultural studies aiming to improve crop yield. Many plants investigated in studies shown in Table 1 are annual grass crops, but little information is available for perennial crops. Perennial plants play an important role in many regions, where poor soil quality only allows for agroforestry as a sustainable farming practice (Lehmann and Joseph 2015). Compared to annual plants, perennials differ in water and nutrient cycling, and therefore, they may be affected differently by biochar. Not many studies have investigated the effects of pure biochar on plant growth and no study performed a comprehensive analysis of metal/metalloid speciation changes.

Accordingly, to address the mentioned knowledge gaps, our study aims to investigate whether biochar is able to improve the fertility of a nutrient-poor and acidic soil in subtropical conditions and in an agroforestry setting with the following local perennial crops: manioc (*Manihot esculenta*), bananas (*Musa* sp.) and papaya (*Carica papaya*). To achieve our objectives, we measured biochar-induced metal mobility in soil, plant metal uptake, and changes in soil microbiology over an 11-month period.

2 Materials and methods

2.1 Field site characterization and biochar production

The study site (AgraDEço experimental farm, 23.3013°S, 44.6916°W) is located in Southeastern Brazil in the Atlantic forest region (Mâta Atlantica, Fig. 1) and lies in a hilly terrain near the city of Paraty (state of Rio de Janeiro, Brazil). The existing farmland in this area and the surrounding virgin forest are stressed by the slash-and-burn management of neo-traditional farming. The inclined terrain and poor soil quality pose additional challenges to local farmers (Begossi 1998) and represent a typical situation in subtropical countries, where new strategies are needed to increase agricultural yields in already deforested subtropical forest habitat. The dominant soil type in this region is orthic ferralsol (van Wambeke 1974), a soil type that is often deficient in P, Ca, Mg, and Mo, but enriched in Al, Mn, and Fe to levels that are toxic for a number of crops. A soil profile was prepared at the experimental site and selected features from the soil profile were identified by X-ray diffraction (cf. section soil and biochar samples characterization).

Biochar was produced from the wood of *Tibouchina arborea* (local name: *Quaresmeira*), which is a common tree abundant at the study site found in secondary growth forests in the area and is documented to be used for char production by indigenous communities (Lorenzi 2002). Pyrolysis was performed in an on-site artisanal-style oven made

of bricks and employing natural airflow control (Fig. S1), which yielded an average pyrolysis temperature of 330 °C (Fig. S2) as recorded using a high-temperature probe (Fisher Scientific). We chose this relatively simple setup, because we wanted to use tools available in the study region, and not to use a source biomass or pyrolysis conditions that are not achievable by the local population. The average duration of pyrolysis was 6 h (4–7.5 h) per loading. At the end of the pyrolysis of each batch, the blaze was quenched with fresh creek water (see next section) and left to cool and dry. The final biochar was then ground in a concrete bowl using a wooden mortar to a particle size of < 1 cm (Fig. S1). The particle size distribution of the produced and ground biochar was analyzed in the laboratory using stainless steel sieves (2 mm, 1 mm, and 0.075 mm).

The experimental fields (slope 24.4%, orientation SWS) were cleared of plants and larger stones, and the soil was broken up using a hand hoe. Three field sites (6.24 m^2) were set up (Fig. S3). The first field (T1) was used as a control, without amendment. The second field (T2) was amended with biochar by applying 2 kg/m^2 (20 t/ha) ground biochar based on Petter and Madari (2012), which was worked into the topsoil using a hoe and a rake to a depth of approximately 15 cm. For the third field (T3), the same amount of biochar was used; however, the biochar was first soaked in urine (0.7 kg/1 kg biochar) and left to soak overnight. This treatment was done to investigate whether additional spiking of the biochar with inorganic nutrients produced additional effects on the ferralsols. Urine treatment was previously shown to enhance crop growth through activation of biochar and enrichment of the soils with nitrogen and metals (Schmidt et al. 2015). Each field was planted in January 2018 in an ordered manner (Fig. S3) with 24 papayas as seeds, 24 manioc plants as cuttings, and 3 bananas as rhizomes. At the end, each field was loosely mulched with dried litter (mostly bamboo leaves) as a protection measure against sunlight and erosion.

2.2 Field sampling (biochar, soil, worms, plants)

Representative samples of biochar and urine-amended biochar were taken prior to mixing with the soil and placed into acid washed 250 mL polypropylene bottles. Soil samples were collected in the same manner just prior to planting. The topsoil of each prepared field was randomly sampled at six locations and mixed together before being placed into the sampling bottles.

Initial plant growth was recorded 28 days after planting, and second sampling of soil from the fields was done in March 2018 (after approximately 2 months). For this, the mulching material was raked aside and the surface soil was collected as in the previous sampling. A final soil sampling was done in December 2018 (after approximately 11 months). At the same date, the plants that survived were counted again and the following growth parameters were measured: total plant height, height of stem from the ground, total number of leaves, and total number of stems (for manioc). In addition, in each field, three earthworm-count measurements were performed by hand-sorting similar to Callaham and Hendix (1997) and FiBL (2017), methods that minimize soil contamination in order that fields are not altered for forthcoming experiments. For this purpose, a 20 cm deep pit with a surface area of approximately 530 cm^2 was dug using a shovel and the extracted shovel volume (estimated 5 L) was hand-sorted. Earthworms were captured, counted, and set free again.

From each banana tree, the first 30 cm of the highest opened leaf were sampled. In addition, a 10 cm long and 2 cm deep piece from the bottom of the main rhizome of each banana plant was sampled (see example in Fig. S4). For manioc, five plants were taken at random from each field and separated into leaves, stems and roots, as shown in Fig. S5. The plant samples were thoroughly flushed with river water prior to being shipped to the University of Alberta for further processing. Surface water was sampled in January 2018 from a small, perennial creek uphill of the experimental fields. Conductivity and pH were recorded in situ using a portable multimeter (OAKTON PCTSTestr 50).

2.3 Soil and biochar samples' characterization

Biochar and soil samples were dried at 104 °C overnight and moisture content was determined by weight loss. Dried samples were ground using a mortar and pestle and sieved to < 2 mm. Total carbon (TC), total organic carbon (TOC), and total nitrogen (TN) were determined by a dry combustion method (Nelson and Sommers 1996) with a Flash 2000 Organic Elemental Analyzer (Thermo Scientific) and atropine as a standard. TOC was calculated by subtracting the total inorganic carbon portion (measured after acidification with 1 M HCl) from the TC. The oxygen and hydrogen contents of the biochar were determined by a Flash HT Plus (Thermo Scientific) and using cyclohexanone-2,4-DNPH as standard. The mineralogy of the untreated soil, biochar, and samples from the B horizon was determined by X-ray diffraction (XRD; Ultima IV, Rikagu) with a cobalt X-ray source ($\lambda = 1.790260$). Interpretation was done using the JADE 9.5 software package and the databases: 2013 ICDD, 2015-1 ICSD.

Another set of samples were air-dried overnight and the pH of each was measured after suspension in ultrapure water for 30 min, and using a 1:2 mass:volume ratio for soil samples (Kalra 1995) and a 1:5 ratio for biochar samples (Jones et al. 2011). Cation exchange capacity (CEC) was determined by the exchangeable cation addition method, as described in Hendershot and Duquette (1986). For this

purpose, 1 g of air-dried sample was suspended in 50 mL of 0.1 M $BaCl_2$ for 2 h. After centrifugation (2000*g*, 10 min), the supernatant was filtered (0.2 µm) and analyzed for Na, Mg, Al, K, Ca, Mn, and Fe. Aluminum was considered to be in the Al^{3+} form and Fe in the soluble Fe^{2+} form. Exchangeable acidity (i.e., $[H^+]$) was determined by measuring the pH. The CEC was then calculated according to $CEC = [H^+] + [Na^+] + [K^+] + [Mg^{2+}] + [Ca^{2+}] + [Mn^{2+}] + [Fe^{2+}] + [Al^{3+}]$ and expressed in cmol (centimole) positive charge/kg dry soil sample (Stuanes et al. 1984).

Surface water samples, soil and biochar digestions, and extraction solutions (see below) were analyzed for metal concentrations using an Agilent 8800 Triple Quadrupole ICP-MS with Ar as a carrier gas, He and H_2 as collision gases, and O_2 as a reaction gas for S, P, and As. ^{45}Sc, ^{74}Ge, ^{115}In, and ^{175}Lu were used as internal standards (Sakai 2015). Concentrations of anions in the water sample were determined by colorimetry (chloride, nitrate, nitrite, phosphate, and sulfate) using the EPA/600/4-79/020 method and by titration with HCl (total alkalinity).

The surface functionality of the produced biochars was examined using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy (Platinum ATR, Bruker). Air-dried sample powders were further reduced in size by an agate mortar and pestle to yield powders that could be pressed between the crystal and the pressure tower. The spectra were recorded as overlays of 16 co-added scans at a resolution of 4 cm^{-1}, in triplicate. The OPUS software interface (v. 7.2) was used for baseline correction of the spectra.

Sequential extractions were performed to assess the metal and metalloid speciation. The extraction was performed according to the Community Bureau of Reference method by Quevauviller et al. (1993) and as applied for biochar by von Gunten et al. (2017). Briefly, 0.5 g of 105 °C-dried sample was used and extracted with solutions of increasing chemical aggressiveness, which allowed the differentiation between the metal/metalloid fractions, including: (1) exchangeable/acid soluble; (2) reducible; (3) oxidizable; and (4) residual. Details on the method are given in the supplementary information.

2.4 Plant matter analysis

All plant parts were dried at 60 °C, cut into small pieces and ground up using a mortar and pestle. The total dry leaf mass of the sampled manioc plants was recorded. Dried plant material was then digested similar to Huang and Schulte (1985). Pre-digestion of 0.5 g of each sample with 10 mL of 70% nitric acid was performed at 60 °C in 50 mL polypropylene centrifuge tubes. After 1 h, the samples were left to cool and 3 mL of 30% hydrogen peroxide were added. After the frothing ended, another 3 mL of hydrogen peroxide were added and the samples were heated at 150 °C until a small amount of liquid remained, which was left to cool and then diluted with 2% nitric acid and 0.5% hydrochloric acid to 50 mL. The liquids were filtered using nylon syringe filters (0.45 µm, Agilent) prior to analysis by ICP-MS.

Because manioc leaves lost their coloration during sample transportation, only banana leaves were used to estimate the chlorophyll content. For this purpose, chlorophyll extraction by dimethyl sulphoxide (DMSO), similar to Arnon (1949) and Hiscox and Israelstam (1979), was performed. 20 mg of each leaf were ground up using an agate mortar and pestle together with 2 mL of DMSO (Fisher Scientific). After all the leaf matter lost its coloration, the liquid was transferred from the mortar into a 2 mL Eppendorf centrifuge tube and centrifuged at 14,000 g for 1 min. The DMSO was then decanted into a UV–Vis cuvette. The absorbance of the sample at 663 nm and 645 nm were measured using an Evolution 60S UV–Vis Spectrophotometer (Thermo Scientific) and the chlorophyll content was estimated using the total chlorophyll equation as given by Arnon (1949).

2.5 Soil microbiology

Microbial DNA was isolated in the laboratory from wet soil samples collected in January, March, and December 2018. The FastDNA™ SPIN Kit for soil (MP Biomedicals) was used (using about 50 mg of soil) and 16S rRNA was amplified by the polymerase chain reaction (PCR) using bacterial and archaeal primers for the V4 hypervariable region (Caporaso et al. 2012). Sequencing was performed using the Illumina MiSeq platform with the Illumina NexteraXT library preparation kit. For data processing, MetaAmp (v. 2.0, Dong et al. 2017) and R (v. 3.4.1) with the PHYLOSEQ package (McMurdie and Holmes 2013; R Core Team 2017) were used. Operational taxonomic unit (OTU) clustering was performed at the 97% similarity level; singletons and unknowns were removed. Selected sequences were compared to the NCBI database using the BLAST tool (Altschul et al. 1990) and only considering matches with 100% query coverage. METAGENassist (Arndt et al. 2012) was used to predict metabolisms by comparable metagenomics. Rowwise normalization was applied in METAGENassist to make samples comparable to each other.

2.6 Statistical analyses and data visualization

Graphs and statistical analyses of plant growth and metal uptake parameters were done in OriginPro (v. 8.6) and R studio (v. 3.5.2). The homogeneities in the population variances were assessed using Lavene's test. In the case inhomogeneous variances, Welch's correction was applied to the one-way independent analysis of variance (ANOVA) with subsequent Games–Howell post hoc testing instead of Tukey post

Table 2 Water content, pH, and CEC of biochar and soil

	Bulk chemistry					Contribution to CEC								
	Water	TC	TOC	TN	pH_{H_2O}	CEC_{total}	Na^+	Mg^{2+}	Al^{3+}	K^+	Ca^{2+}	Mn^{2+}	Fe^{2+}	H^+
	wt%	wt%	wt%	wt%		cmolc/kg	%	%	%	%	%	%	%	%
Jan-18														
Biochar1	55	82	80	*N/D*	9.71 ± 0.06	44.0 ± 0.7	8	7	0	10	75	0	0	0
Biochar2	75	82	82	*N/D*	9.44 ± 0.03	30 ± 6	45	7	0	45	3	0	0	0
HorB	19	0.6	0.6	0.07	4.04 ± 0.13	2.14 ± 0.16	0	4	69	1	12	0	0	13
Control	23	3.7	3.6	0.27	4.77 ± 0.07	4.45 ± 0.17	0	18	14	7	57	0	0	5
Mix1	33	8.8	8.7	0.32	6.34 ± 0.09	7.6 ± 0.4**	1	20	0	8	70	1	0	0
Mix2	28	4.8	4.8	0.25	6.53 ± 0.10	8.6 ± 1.5	5	17	0	12	65	0	0	0
Mar-18														
Control	27	3.3	2.9	0.24	4.67 ± 0.06	4.22 ± 0.18	0	16	15	4	60	1	0	4
Mix1	36	7.8	6.3	0.33	5.30 ± 0.13*	8.4 ± 1.2	0	20	3	3	72	1	0	1
Mix2	30	4.4	4.4	0.21	5.16 ± 0.01*	5.5 ± 0.5	0	17	8	6	65	1	0	3
Dec-18														
Control	21	3.9	3.7	0.29	4.56 ± 0.09	3.4 ± 0.4	1	18	21	6	46	0	0	8
Mix1	34	6.0	5.5	0.30	5.04 ± 0.01*	5.33 ± 0.10*	2	19	7	5	64	0	0	3
Mix2	28	4.0	3.5	0.25	4.92 ± 0.02*	4.94 ± 0.02*	1	18	12	5	59	0	0	4

Cation fractions in mol% show the relative contribution of metals to the soil CEC. Mix1: field with bare biochar. Mix2: field with urine-amended biochar. Where available, data given as average ± standard deviation ($n = 3$). Statistical significance of changes in the mix1 and mix2 fields compared to the control of the same date is indicated with asterisks: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. *N/D* not detected

hoc pairwise comparison. The significance level α was set at 0.05. To compare microbiological communities between the fields, Analysis of Molecular Variance (AMOVA) was performed using mothur (v. 1.39.5) (Dong et al. 2017).

3 Results

3.1 Soil characteristics

The soil profile from the field site is shown in Fig. S7. The A horizon (up to 15 cm), which was affected by the biochar treatments, consisted of decaying organic matter, and contained the minerals quartz (SiO_2), gibbsite ($Al(OH)_3$), kaolinite ($Al_2(Si_2O_5)(OH)_4$), and microcline ($K(AlSi_3O_8)$). The underlying B horizon consisted mainly of quartz, gibbsite, microcline, dickite (a polymorph of kaolinite), and augite ($(Ca,Na)(Mg,Fe,Al,Ti)(Si,Al)_2O_6$). Several localized accumulations were found in the horizon B, such as quartz/halloysite, gibbsite/hematite, quartz/goethite/phlogopite (Fig. S7). The pH of the A horizon averaged 4.8, whereas the B horizon had a pH of 4.0 (Table 2). The CEC of the A horizon was approximately twice as high as in the B horizon, with 4.5 cmolc/kg, similarly low as in the study by Steiner et al. (2007). While the CEC of the A horizon was mainly controlled by Ca and Mg (57% and 18%, respectively), the CEC of the B horizon was mostly attributed to Al (69%) (Table 2).

The A and B horizons were rich in Al, Fe, K, and Ca, with up to 71 mg/g, 15 mg/g, 9 mg/g, and 2 mg/g, respectively (Table 3). Abundant trace metals were Ba, S, Mg, P, and Mn. Horizon A was generally more concentrated in trace metals with the exception of Ni, Sr, Cd, Ce, and Th. Sequential extractions of the B horizon revealed that with the exception of Mg, Ca, and Mn, the majority of major elements were rather immobile, being found mostly in the residual, oxidizable, and reducible fractions (Fig. 2). 87% of Mg, 37% of Ca, and 32% of Mn were in the exchangeable/acid-soluble fraction (i.e., Mn^{2+}), suggesting high potential mobility. This was similar to the A horizon (Fig. 2, control field, 0–11 mt), where Mg, Ca, and Mn were equally mobile with 50–57%, 2–26%, and 2–31% being in the exchangeable/acid-soluble fraction, respectively.

Water from the nearby creek reflected the soil chemistry, having a pH of 5.7 and low concentrations of all metals with a resulting conductivity of only 55 µS/cm (Table S1). The latter suggests that the dissolution of soil components by surface runoff is minimal.

3.2 Biochar characteristics

The biochar primarily consisted of smaller particles of 0.075–1 mm size (59%), followed by particles > 2 mm (19%), 1–2 mm (18%), and < 0.075 mm (3%). XRD measurements showed that the biochar contained quartz, calcite ($CaCO_3$), halite (NaCl), and traces of talc ($Mg_3(Si_4O_{10}(OH)_2)$) (Fig.

Table 3 Bulk properties of soil, wood, and biochar

Elements	HorA (Control)	HorB	Wood	Biochar1	Biochar2
Ash content (%)	*N/M*	*N/M*	1	16	10
Na (mg/g)	*N/M*	*N/M*	0.51 ± 0.12	30.9 ± 0.2	29.23 ± 0.12
Al (mg/g)	69 ± 6	71 ± 4	0.37 ± 0.08	4.52 ± 0.08	2.39 ± 0.03
K (mg/g)	5.9 ± 0.6	9.3 ± 0.9	0.74 ± 0.02	4.29 ± 0.06	6.44 ± 0.10
Ca (mg/g)	2.2 ± 0.3	1.6 ± 0.2	1.4 ± 0.4	12.52 ± 0.06	7.18 ± 0.05
Fe (mg/g)	12.8 ± 1.8	15.0 ± 0.9	0.04 ± 0.01	0.958 ± 0.019	0.38 ± 0.05
B (µg/g)	250 ± 80	150 ± 60	4.8 ± 1.1	100 ± 10	190 ± 40
Mg (µg/g)	540 ± 50	120 ± 30	400 ± 80	2010 ± 20	1350 ± 17
P (µg/g)	250 ± 30	101 ± 6	59 ± 8	347 ± 5	1005 ± 3
S (µg/g)	380 ± 30	270 ± 40	170 ± 30	703.1 ± 0.3	1040 ± 11
Mn (µg/g)	230 ± 20	161 ± 17	2.2 ± 0.7	75.8 ± 0.7	47.1 ± 1.3
Ni (µg/g)	2.69 ± 0.19	4.3 ± 0.5	0.178 ± 0.014	3.7 ± 0.7	2.8 ± 0.4
Cu (µg/g)	3.7 ± 0.3	1.8 ± 0.3	2.4 ± 0.2	44 ± 6	32.2 ± 1.4
Zn (µg/g)	270 ± 130	60 ± 60	2.06 ± 0.20	170 ± 10	200 ± 60
Sr (µg/g)	26 ± 3	33 ± 4	13 ± 3	78.5 ± 0.7	50.25 ± 0.16
Mo (µg/g)	3.5 ± 0.3	3.4 ± 0.4	0.040 ± 0.004	10.50 ± 0.04	22 ± 5
Ba (µg/g)	170 ± 30	280 ± 60	26 ± 6	280 ± 60	350 ± 50
Pb (µg/g)	41 ± 3	40 ± 30	0.87 ± 0.07	9 ± 1	6.54 ± 0.17
H (wt%)	*N/M*	*N/M*	*N/M*	2.39	2.38
O (wt%)	*N/M*	*N/M*	*N/M*	0.17	0.32

HorA refers to the horizon A (equal to control field top soil). HorB: horizon B. Biochar1: pure biochar. Biochar2: urine-amended biochar. Note the different units for concentrations. *N/M* not measured

S8). The amended biochar had a similar mineralogy, but also contained sylvite (KCl), likely contributed by the addition of urine (Kirchmann and Pettersson 1994). The high abundance of alkaline elements (e.g., K, Mg, and Ca) and calcite in both biochar samples likely contributes to the high pH of the biochar (Singh et al. 2010), ranging between 9.4 and 9.7 (Table 2). Such high values are typical for wood-derived biochar (von Gunten et al. 2017).

The molar O:C values of the biochar samples were very low (0.002–0.003) suggesting low hydrophilicity (less oxygen-containing groups) and high stability in soil (Spokas 2010; Shen et al. 2012) (Table 3). Molar H:C ratios (0.347–0.350) are indicative of high aromaticity of the samples (Shen et al. 2012), which would further imply high resistance towards bacterial degradation. The N content of the biochar was very low (< 0.01 wt/%) and not affected by the urine amendment (Table 3). It is likely that nitrogen contained in the urine amendment evaporated in the basic biochar environment during the soaking process, probably as NH_3 (Cameron et al. 2013).

The CEC of biochars 1 and 2 was nearly ten times higher than that measured in the A horizon of the soil, with 44 cmolc/kg and 30 cmolc/kg, respectively (Table 2). In the case of biochar 1, 75% of the CEC were controlled by Ca, while the CEC of biochar 2 was dominated by K and Na (both 45%), likely reflecting the effect of the urine amendment.

The metal composition of the biochar samples was in general comparable to other wood-derived biochar (von Gunten et al. 2017). Both samples were rich in Ca, K, Al, Mg, S, and P, with up to 13 mg/g, 6 mg/g, 5 mg/g, 2 mg/g, 1 mg/g, and 1 mg/g, respectively (Table 3). The chemistry of the biochar can be explained by the composition of the *Tibouchina* wood, as comparatively visualized in Fig. S9. While the relative distribution in wood and biochar is similar, the pyrolysis process concentrated all metals by factors of 4–93, with average factors of 33 and 42 for biochars 1 and 2, respectively. This affected primarily the lighter elements abundant in wood, such as Zn, Na, B, Li, V, Cr, and Mn. Compared to biochar 1, biochar 2 had higher concentrations of B, P, S, K, and Mo, with biochar 2/biochar 1 metal ratios of 1.8, 2.9, 1.5, 1.5, and 2.1, respectively. On the other hand, many major metals (e.g., Mg, Al, Ca, and Fe), as well as some trace metals (e.g., V, Cr, and Mn), had lower concentrations in biochar 2. Many elements in the biochar samples showed elevated potential mobility, having a high exchangeable/acid-soluble fraction (Fig. 2). Notable examples were Mg, P, S, K, Ca, Mn, and Zn, with up to 59%, 61%, 77%, 84%, 80%, 67%, and 33%, respectively, being in the exchangeable/acid-soluble fraction.

ATR-FTIR spectra of the biochars (Fig. S8) indicated that the carbonaceous structure of the biochars contains both alkane and alkene functional groups as indicated by the sp^3 and sp^2 C–H stretches, respectively (Song et al.

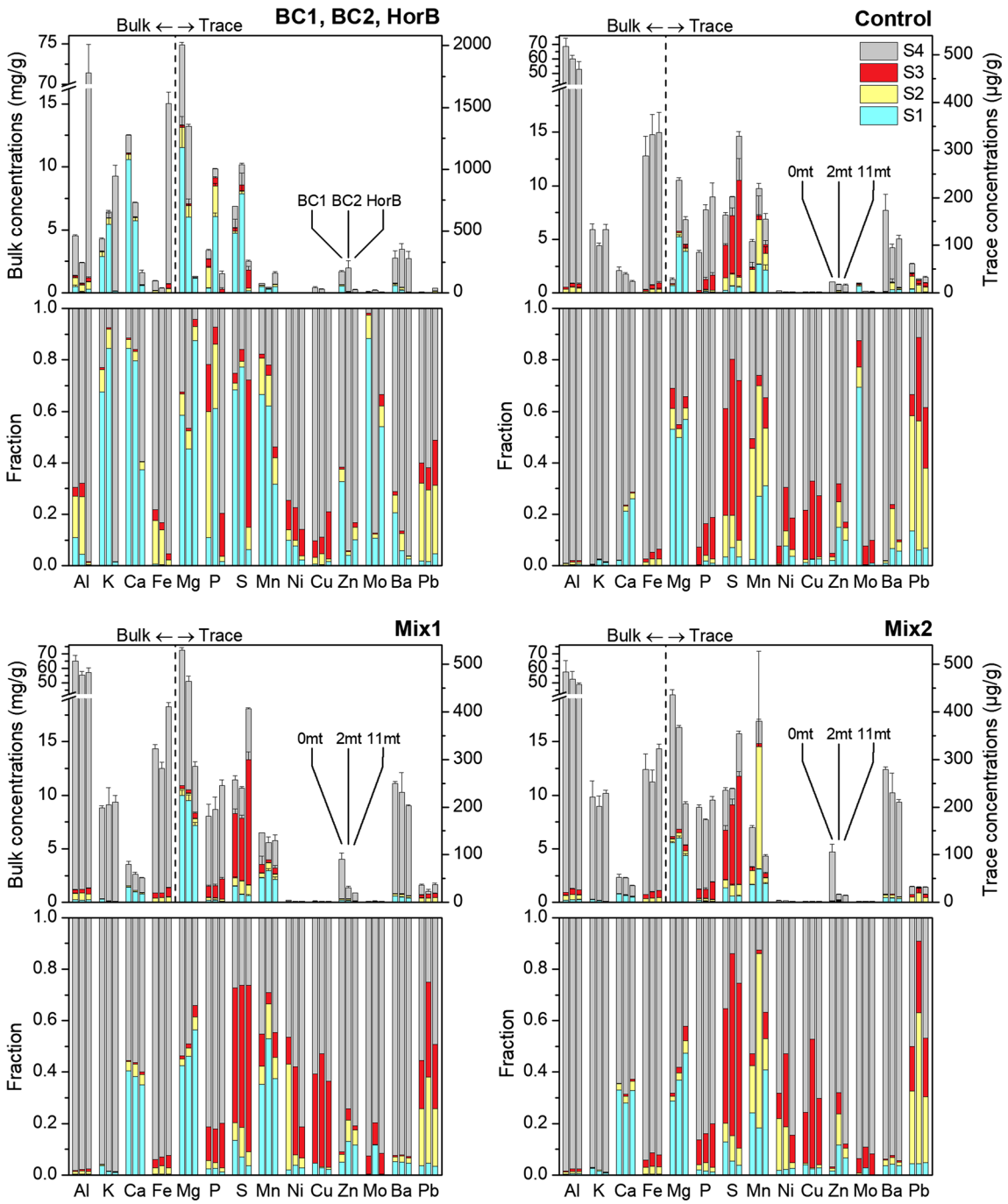

Fig. 2 Sequential extraction results (absolute and relative). The top left graphs represent the composition of biochar 1 (BC1), biochar 2 (BC2), and the horizon B (HorB). The top right graphs represent the control field at the three sampling times (0 months, 2 months, and 11 months). The bottom graphs show the data for the fields amended with biochar 1 (Mix1) and biochar 2 (Mix2). Note different axes range for bulk and trace elements in the absolute graphs. The four different fractions are (F1) exchangeable/acid soluble, (F2) reducible, (F3) oxidizable, and (F4) residual

2015). The presence of oxygen-containing surface functional groups (OCSFGs) was found particularly strong in a carbonyl (C=O)-stretching band at around 1738 cm^{-1}. Additional sp^2 C–O-bending vibrations between 1300 and 1200 cm^{-1}, and possibly between 1150 and 1000 cm^{-1} (sp^3 C–O), could indicate the presence of esters and/or ether functional groups. In addition, the absorption between 1550 and 1350 cm^{-1} is consistent with carbonates, supporting the XRD findings. This is further corroborated by the C–O-bending vibration at around 872 cm^{-1}, which is

characteristic for $CaCO_3$. The bands at around 1084 cm^{-1}, together with the weak doublet at 797/779 cm^{-1}, would substantiate the presence of quartz, while the absorption at around 1000 cm^{-1} could indicate the presence of sulfates, as suggested by the digestions (see below).

3.3 Changes in soil geochemistry

3.3.1 Initial soil geochemistry

Amendment with biochar immediately increased soil pH from pH 4.8 to 6.3–6.5 (Table 2). Similarly, biochar application changed the CEC in the soil, with a significant change for the mix1 field (Table 2). The Al contribution to CEC decreased from 14% to zero and was compensated by increases in Na, K, and Ca. Introducing biochar into the topsoil increased the TOC contents of the fields (Table 2).

Application of biochar initially increased the abundance of K, Mg, P, S, Mn, Zn, and Ba in both test fields (Fig. 2). The most notable increase was for Mg with factors of 18 and 15 in mix1 and mix2, respectively, as compared to the control field. This was followed by Zn and Ba, with factors of 4 and 5 for mix1 and mix2, respectively, likely due to the high abundance of those elements in the biochar (Table 3). Phosphorus increased by a factor of 2, while K increased by factors of 1.5 and 1.7 for mix1 and mix2, respectively. A notable decrease was found for Pb, where the total concentration was lower by a factor of 0.6 and 0.5 for mix1 and mix2, respectively, compared to the control field. This represents a "dilution effect" in the topsoil following the application of the light biochar. Biochar amendments did not change substantially, with the concentrations of Ni being around 4 µg/g in all fields.

In the biochar-amended fields, some major elements (e.g., K, Ca, P, and S) had greater exchangeable/acid-soluble fractions compared to the control field (Fig. 2). This is possibly a result of their abundance and mobility in the biochar. The exchangeable/acid-soluble fraction of K increased by up to 8.4 times; however, this first fraction remained relatively small in relation to the others (< 4%). Calcium concentrations increased up to 1.6 times upon amendment with biochar and its exchangeable/acid-soluble fraction increased by up to 19 times. In the biochar fields, P and S initially appeared to be more mobile with exchangeable/acid-soluble fractions, being 18 and four times higher, respectively. Marginal or opposite changes were observed for Mg, Fe, Ba, Cu, Ni, and Pb. The exchangeable/acid-soluble phase of Mg was high in all fields in the beginning, ranging from 29–53%. Manganese showed high variation, but was generally more concentrated in the biochar fields with 146–157 µg/g compared to 108 µg/g in the control field.

3.3.2 Changes in soil geochemistry after 2–11 months

Two months following application, the field site had experienced an estimated 700 mm of rain at an average temperature of 21 °C. After 11 months, the same field site experienced approximately 2600 mm of rain at an average temperature of 20 °C (Fig. S10). At the end of the experiment, biochar particles were still visible in the soils and color differences between control fields and biochar-amended fields remained observable (Fig. S14).

Measurements of pH of samples collected after 2 months showed that the initially strong pH-effect declined over time. Two months after application, the biochar fields had pH values of 5.2–5.3, compared to 4.7 in the control field. However, the pH remained elevated even 11 months post-application. Compared to the control (pH 4.6), the biochar-amended fields remained at pH 4.9 and 5.0, suggesting a long-lasting biochar effect. In addition, the soil CEC remained high after 2 and 11 months, but the Al contribution to the total CEC increased again after 11 months, decreasing the Ca and K fractions to their initial fractions as per the control field.

The increase in TOC upon biochar amendment was still detectable after 2 months in both the biochar-amended fields, having concentrations between 4.4 and 6.3 wt%. While the mix1 field still had elevated TOC concentrations of 5.5 wt% after 11 months, the mix2 field decreased to 3.5 wt%, a value similar to that of the control (3.7 wt%).

Exposure to the weather led to an overall decrease in the measured mobility of K, suggesting losses by leaching. For Ca, the exchangeable/acid-soluble phase remained high over time. The highest changes in mobility were observed for Mg. While the exchangeable/acid-soluble fraction of Mg remained similar in the control field over time, it increased in the two biochar fields. This suggests that mobile Mg was contributed by the added biochar, likely being slowly liberated from its most mobile Mg fractions (exchangeable/acid soluble and reducible) upon weathering and plant growth. Although P and S were more mobile initially in the biochar-amended fields, their mobile fractions became similar to the control after 11 months. While the Mn concentration remained high in the control field, it was lower in the biochar fields after 11 months, at 97–130 µg/g. At the end of the trial, 31–41% of the Mn in the three fields was in the mobile exchangeable/acid-soluble fraction, with a large, highly immobile fraction (35–45% residual). Zinc and Ba remained relatively immobile over time, having less than 7% in the exchangeable/acid-soluble fraction in the biochar-amended fields.

3.4 Plant performance

Initial growth results obtained after 1 month (counts of plants survived) did not indicate clear differences between

Table 4 Results of the visual growth inspection of the experimental fields (number of living plants)

Field	Control	Mix 1	Mix 2
Growth after 1 month			
Papaya	15/24	22/24	21/24
Manioc	23/24	24/24	24/24
Banana	1/3	3/3	1/3
Growth after 11 months			
Papaya	0/24	0/24	0/24
Manioc	15/24	20/24	18/24
Banana	3/3	3/3	3/3

survive the returning acidity or that they were overgrown by weeds.

While the results for manioc in terms of plant survival after 11 months are not conclusive, we measured statistically significant increases in number of leaves, maximal plant height, stem height, and total leaf mass (dry) (see Fig. 3). In the two biochar fields, the number of leaves, plant height, stem height, and total leaf mass increased on average by factors of 1.7 and 1.8, 1.4 and 1.6, 1.5 and 1.7, and 11 and 13, respectively. Although plants in the biochar-amended fields appeared to be able to form several stems, the determined changes were not significant (Fig. S11). No plants showed signs of nutritional problems.

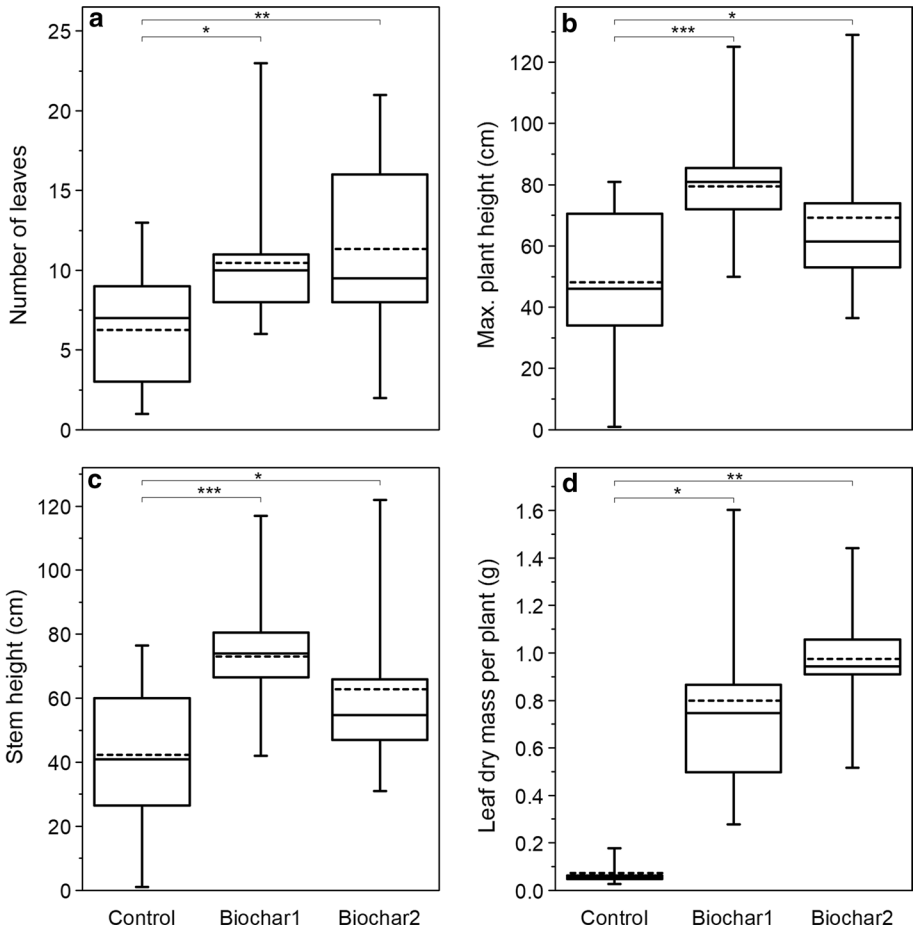

Fig. 3 Box plots show the mean, lower (Q1) and upper (Q3) quartiles, and minimal and maximal values of different properties related to plant growth ($15 \le n \le 20$ for **a**–**c**, $n = 5$ for **d**). Median and mean values are indicated with solid and dashed lines, respectively. Improved plant growth was manifested in a statistically significant increase of those properties upon amendment with biochar. Statistical significance is indicated with asterisks: $*p \le 0.05$, $**p \le 0.01$, $***p \le 0.001$

the three fields (Table 4). Nonetheless, *C. papaya* generally performed better in the biochar-amended soils compared to the control, which can be attributed to the plant's preferred soil pH range of 6–7 (Ikeda 1976) and the buffering effect of the biochar amendment. In contrast, *Musa* sp. and *M. esculenta* both performed well in acidic soils (Islam et al. 1980; Gogoi et al. 2004). No papaya plants survived after 11 months. It is possible that the papaya seedlings did not

All monitored phenological changes of the banana plants were insignificant (Fig. S11), including the change in the chlorophyll content in the banana leaves, having on average 1.9–2.0 mg total chlorophyll per g fresh leaves. Although the average number of earth worms in the biochar-amended fields was higher on average with 13 worms per shovel volume (246 worms/m^2) compared to an average of 7.7 worms per shovel volume (145 worms/m^2) in the control field, this

difference was not statistically significant (Fig. S11). As with the manioc, no banana plants showed signs of nutritional problems.

3.5 Elemental uptake of plants

Elemental concentrations in banana plants were only statistically different between the control group and the biochar groups for Cu. The concentration of Cu in banana roots from the mix1 field was twice as high as in the control field (Table S3). Comparison among manioc roots indicated statistically different concentrations for Mn, which decreased by a factor of 2 upon biochar amendment (Fig. 4). For the stems, Mg and Mo concentrations were significantly increased in mix1 compared to the control field, while concentrations of Cu and Pb were decreased in both biochar fields. Biochar-induced effects in metal concentrations were more evident for the manioc leaves. Similar to the stems, an increase for Mg (from control to mix1) was observed. For the urine-amended biochar field, the concentration of K was significantly higher compared to the control. For the majority of the measured trace metals, decreases in concentrations were observed compared to the control group. For Mn, Fe, Ni, and Cu, leaf concentrations decreased by up to 4, 3, 12, and 2 times compared to the control group. Because all of the leaves were sampled from each manioc plant, the total metal uptake (Fig. 4) for the leaves was calculated using the total dry mass of leaves (Fig. 3) and the leaf concentrations. This showed significantly higher total metal content for all elements as expected given the higher total dry mass due to enhanced growth.

3.6 Changes in soil microbiology

The microbial communities of the test fields were dominated by the classes Alphaproteobacteria, Planctomycetacia, Gammaproteobacteria, and Verrumicrobiae (Fig. S12). The composition was similar between the three fields and between the 3 tested sampling times, with differences not being significant as determined by AMOVA ($p > 0.1$). Alphaproteobacteria was mostly represented by OTUs of the *Xanthobacteriaceae* family, such as *Rhodoplanes* and *Bradyrhizobium* (Table S4). While *Rhodoplanes* is a nitrate reducing photosynthetic bacterium (Hiraishi and Ueda 1994), *Bradyrhizobium* is a nitrogen fixing bacterium living in symbiosis with plants of the *Fabaceae* family and being tolerable of high acidity and high Al environments with periodic droughts (Bottomley 1992). The most abundant Planctomycetacia OTU was similar to *Planctomyces maris* (KF228168.1), as determined by BLAST search. *Planctomyces* is a widely present genus with some unique features, such as compartmentalization similar to eukaryotic cells (Fuerst et al. 1997; Scheuner et al. 2014). Important Gammaproteobacteria OTUs were similar to *Acidibacter* and *Massilia*. *Acidibacter* is an acidophile iron-reducing bacterium able to tolerate high Al concentrations (Falagán and Johnson 2014). *Massilia* is a root-associated bacterium, which reacts strongly to plant development (Ofek et al. 2012). Not surprisingly, its abundance showed high variability over the sampling times in all three fields, ranging from 1.0% to 3.1% of the community at timepoint zero to 0.1–0.3% after 11 months.

The overall species richness and diversity were similar in all three fields at timepoint zero (Fig. 5). In the control field, both parameters decreased over the first 2 months (to about 82% of the initial value), while they increased in the biochar-amended fields to more than 123%. The species richness rehabilitated again after 11 months in the control field to nearly the same value as before, while in the biochar fields, it dropped slightly again to 114–117% of the initial value. The species diversity, on the other hand, increased in all three fields after 11 months, with the two biochar fields demonstrating the highest diversity with 265% and 175% of the initial values for the biochar1- and biochar2-amended fields, respectively.

Predicted metabolisms (by METAGENassist) suggest that certain metabolic groups increased over time. Examples shown in Fig. S13 are ammonia oxidizers, xylan degraders, and biomass degraders. On the other hand, nitrite reducers showed a decreasing trend. Biochar applications seem to have affected the abundance of biomass degraders in a positive way. After 11 months, they showed a fivefold increase in abundance compared to the control field at timepoint zero, while in the control field itself, they only increased by a factor of 3. Furthermore, the abundance of bacteria that can degrade aromatic hydrocarbons is predicted to have increased modestly in the biochar-amended fields, with final abundance values being 1.1–1.4 times higher than in the control, while in the control field, the value was the same after 11 months, as it was at the beginning of the experiment.

4 Discussion

4.1 Biochar-induced biogeochemical changes

Biochar amendment increased the pH values of the amended fields for nearly a year, providing the soils with better acid buffering capacity and decreased Al acidity. In addition, biochar was a carrier for important plant macro- and micronutrients, such as K, Ca, Mg, P, S, and Zn. In the case of Ca, its concentration and potential mobility was much higher than in soil. Therefore, wood-based biochar produced on-site with simple pyrolysis methods could be a delivery system for this important plant macronutrient, which benefited plant growth in addition to promoting soil pH favorable to plant growth and aiding in moisture retention. In regard to soil properties

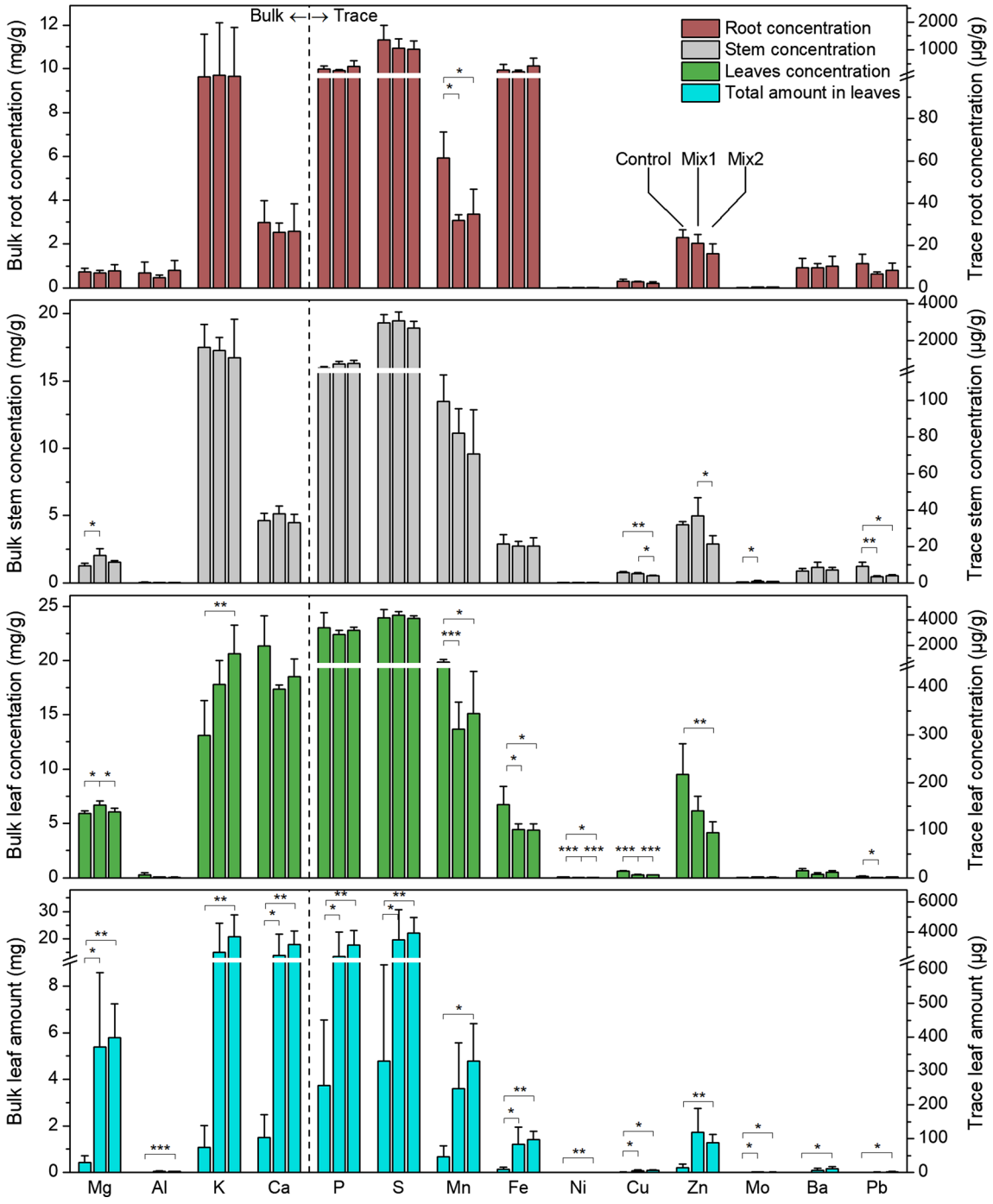

Fig. 4 Metal concentrations/amounts in manioc plant parts. Error bars represent standard deviations ($n=5$). Statistical significance is indicated with asterisks (where applicable): *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. For detailed numbers, see Table S3

and macronutrients, our results are comparable to the study performed in Indonesia by Ismali et al. (2011) using manure biochar and manioc stem biochar. The soil expressed pH, CEC, and concentrations of N, P, K, Ca, Mg, and Al were also in the same order of magnitude as those reported by Steiner et al. (2007) for a similar but less nutrient-deprived soil in Brazil. These comparable outcomes substantiate the versatility of biochar as a soil amendment for a variety of soil and environmental conditions.

In terms of nutrient availability, biochar amendment improved the initial conditions of the soils, as indicated by the increased initial viability of papaya. However, competition with weeds appeared to be a more important factor for papaya than for other studied plants due to the way they were

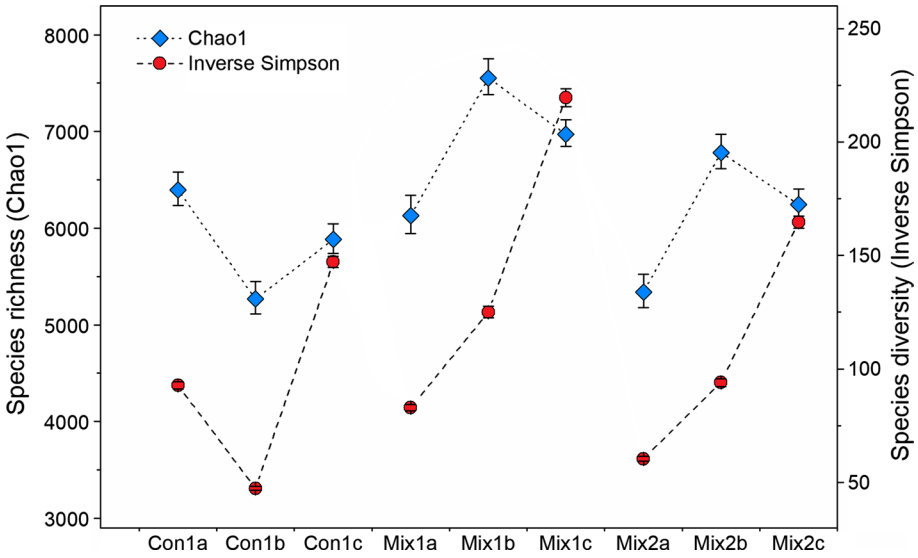

Fig. 5 Species richness (Chao1 index) and species diversity (Inverse Simpson index) determined by 16S rRNA gene sequencing. The values are averages of random subsampling ($n = 1000$). The error bars represent the 95% confidence intervals. Con: control field. Mix1: soil amended with biochar1. Mix2: soil amended with biochar2. a, b, and c represent sampling times: 0 mt, 2 mt, and 11 mt

planted. Weeds not only compete for sunlight but also water and nutrients, for which cycling was modified by the biochar amendments. Unfortunately, the contribution of weeds to elemental uptake was not further investigated in this study.

The soils were initially in a suboptimal state for the growth of manioc, mainly due to low pH (close to the critical levels of pH 4.6) and because of the low potentially mobile concentrations of K, Ca, and Mg (refer to chapter 9 in Howeler 2014). K and P are particularly critical nutrients in ferralsols (van Zwieten et al. 2019). Potassium is a particularly critical nutrient for manioc (Fernandes et al. 2017), and it can become more important than P in South America's subtropical regions (Baligar et al. 2001). Although the biochars had higher fractions of exchangeable/acid-soluble K than the soils, the biochar/soil ratio was too low to measurably increase the absolute amount of potentially mobile K in the biochar/soil mixture. While the biochar/soil ratio might be increased to obtain better K loadings, our extensive metal/metalloid analysis indicates that this ratio as well as the feedstock need to be carefully controlled with regard to release of potentially harmful metals, such as Ba. *Tibouchina* biochar increased concentration of soil Ba, which can be considered a contaminant in wood biochar (Yargicoglu et al. 2015; von Gunten et al. 2017). In the present study, Ba was shown to be rather immobile in the investigated soils (high residual fraction), and therefore, it could potentially accumulate over time if biochar was regularly applied as a soil amendment. Concentrations of P and S increased in all test fields over time, presumably due to plant growth (nutrient transfer from deeper soil layers) and subsequent decay, as well as bioturbation. This is supported by the large oxidizable fractions of those two elements, suggesting their strong association with organic matter (Tessier et al. 1979). Manganese concentrations and availability became smaller in the biochar-amended fields after 11 months, mainly due to a decrease in the reducible fraction, which likely represented easily reducible Mn oxyhydroxides (Tessier et al. 1979). This likely led to decreased potential Mn toxicity, one of the limiting factors in ferralsols (Baligar et al. 2001).

Biochar applications clearly improved manioc growth as reflected in increased plant height (longer stems) and higher leaf biomass. Improved growth conditions in the biochar fields were also corroborated by the observed higher viability after 11 months (Table 4). The increased growth of manioc is likely due to nutrients addition by biochar (e.g., more than 1.5 times increase for K) in combination with decreased soil acidity and metal toxicity stresses (Al, Mn). The yield of manioc's edible tuberous roots remains unknown, as this result was not targeted by this study and would require longer growth times and greater plant spacing under the given conditions (Howeler 2014). Nevertheless, the observed > 60% increase in manioc height and leaf mass corresponds to the > 60% manioc yield increase reported by Islami et al. (2011) when using biochar made from manure or manioc stems. Interestingly, while total metal uptake in leaves in the amended fields was higher for each element analyzed, their concentration in leaves and other plant parts varied. For many elements, the total uptake was lower than what would be expected from biomass increase. It remains to be investigated, whether this effect is a result of altered nutrient uptake or rate of dry matter accumulation.

There was no apparent change in the distribution of major microbial classes in the amended fields (Fig. S12), although there was an increase in species richness and diversity (Fig. 5). The overturning of the soil at the beginning of the experiment likely exposed microbes to UV radiation, which led to a dramatic decrease in species richness and diversity in the control field within only 2 months (Romanovskaia et al. 1999). This did not happen in the biochar-amended fields, potentially due to UV-shielding provided by biochar

particles. Plant growth then increased the UV-shielding in the control field as well, allowing the richness and diversity to recover. Biochar is also known to provide suitable growth substrate for microbes (Zhu et al. 2017), which might have contributed to the species richness and diversity increase. The species richness and diversity rose substantially in the biochar-amended fields over time. Between 2 and 11 months, the richness in the biochar-amended fields began to decline again, suggesting that the emerging biochar-induced communities would eventually return to their initial state. The decline in species diversity in the amended fields was slightly slower compared to the species richness. These declines could suggest biochar degradation and population by bacteria and archaea occurs only to a limited extent. Such an effect might remain undetected in shorter-term field studies (for example, see Simarani et al. 2018).

4.2 Biochar as a potential fertilizer alternative?

The relative distribution of elements in the soil was reflected by the chemistry of the wood and maintained by in the biochar produced thereof (Table 3, Fig. S9). The *Tibouchina* wood used in this study was not particularly rich in P, K, and Ca, which are key nutrients often missing in ferralsols (van Wambeke 1974), but the pyrolysis process and partial ashing concentrated those elements 6–9 times. However, when comparing the pure biochar to a locally produced and commercially available mineral fertilizer, the elemental composition of the biochar is remarkably lower. This is particularly the case for P, which is 214 times more concentrated in the mineral fertilizer, whereas K and Ca are only 16 and 7 times as high, respectively (Table S2).

Amendment by urine only marginally increased the nutrient contents, e.g., factors of 2.9 for P, and 1.5 for S and K compared to pure biochar. In addition, the urine treatment depleted the biochar in metals such as Al and Cr, which may pose plant and human toxicity concerns. Other pretreatments of biochar would be possible. For example, biochar was also used for odor control in a dry-toilet at the experimental site. The biochar–waste mixture was typically quickly inhabited by black soldier fly larvae (*Hermetia* sp., Fig. S15), which resulted in efficient composting of the material. In this way, the recovered biochar was enriched in nutrients (Table S2). As a comparison, P, K, Ca, and N in the mineral fertilizer are only 8, 14, 4, and 3 times higher than in the *Hermetia* biochar, making it a more promising biochar amendment compared to urine as investigated in this study. However, more research would be needed to evaluate risks associated with potential chemical and biological contaminants originating from human and animal excretions when formulating such an alternative to mineral fertilizers.

Advantages of using biochar as compared to the mineral NPK fertilizer is the higher pH (pH 9.7 vs. 3.0) that counteracts the already acidic soil and does not require additional liming if applied. Furthermore, the use of locally produced biochar does not add contaminants to the soil. For example, the NPK fertilizer contains non-negligible traces of potentially harmful metals, including Co, As, Cd, and U, with concentrations of 9 µg/g, 3 µg/g, 2 µg/g, and 26 µg/g, respectively (Table S2). Still, a well-balanced combined treatment (biochar and NPK fertilizer) could be the most promising long-term solution, as shown by Steiner et al. (2007), who found significantly larger crop yields in such cases.

5 Concluding remarks

In the form of terra preta, biochar was likely used for soil improvement for thousands of years by indigenous communities of South America (Petter and Madari 2012). Today, biochar could be a simple and cost-efficient technology for farmers, especially as a low-emission alternative to slash-and-burn land management techniques (Lehmann et al. 2006). In this regard, wood-waste-derived biochar, such as the one used in this study, would be well suited for less harmful and more sustainable soil management in an agroforestry setting. Our study provides a comprehensive overview of biochar effects in sloped orthic ferralsols on metal/metalloid speciation, mobility, and bioaccessibility changes and suggests that biochar applications would not only enrich the soil by critical nutrients, such as K, Ca, and P over a growing season, but also shift the soil biogeochemistry towards a more fertile state for perennials. Potential soil burdening with inorganic (e.g., Ba) and organic contaminants need to be considered and require more applied, long-term studies, especially when considering repeated applications and higher dosage. Despite the lower nutrient concentrations compared to mineral fertilizers, the additional benefits of reduced soil acidity and microbial diversity resulted in significantly increased growth of manioc. Different biochar amendments could be applied to further enhance the plant nutrient content and could, therefore, be a valuable sustainable resource to mitigate deforestation in this Atlantic Forest region.

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