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Long-term impact of single biochar and compost application on soil aggregation

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Abstract. Soil aggregation is an important indicator of soil quality and highly responsive to management such as application of organic amendments. Compost generally increases aggregate stability and enhances soil microbial activity, while the effects of biochar on these factors remain inconclusive. We investigated the effect of biochar and compost on soil aggregation and microbial abundance at an experimental soil rehabilitation site in Ngaoundéré, Cameroon. Sampling was carried out 3.5 years after installation of the site. Both amendments improved bulk density, hydraulic conductivity, pH, and base saturation. Cation exchange capacity and soil organic matter (SOM) content were rather a function of soil texture than influenced by the amendments. Bacterial abundance increased in the compost, but not in the biochar treatment. Fungi were more frequent in smaller aggregates, but did not respond to the treatments. Macroaggregates 400 – 2,000 µm contributed ca. 75 % of the soil functions assessed. Yet, SOM content was 4 times higher in microaggregates < 50 µm than in macroaggregates throughout all treatments. We conclude that single applications of organic amendments can have positive long-term effects on soil aggregation in undisturbed degraded soils, particularly in the macroaggregate fraction. Microaggregates harbour fungal hyphae and are rich in SOM, independent of amendments.

1. Introduction

Soil degradation affects more than one billion people worldwide [1], particularly in dry regions, where around 40% of the world population live [2]. Soil degradation is indicated by a loss of soil functions, a large portion of which depends on soil aggregation and soil organic matter (SOM) storage within aggregates [3,4]. Six *et al*. (2000) report that most soil C is contained in macroaggregates [5] and Six *et al*. (2004) describe microaggregates as the compartment contributing most to soil C stabilisation by occlusion [6]. Aggregate formation follows a specific lifecycle that includes microbial colonisation of interior surfaces and SOM stabilisation in the necromass resulting from dying bacterial micro-colonies [5]. Biochar and compost are effective means to improve both soil aggregation and soil C content [7], and have since found wide agronomic application, particularly in tropical regions. The provision of microbial substrate by compost [3] and of habitat by biochar [8,9] are central in sustainable soil amelioration using co-amendment of biochar and compost [10]. A new field of biochar and compost application is the large-scale rehabilitation of degraded tropical soils to restore their functions and to enable sustainable use over the long term. In the ReviTec® approach for sustainable soil rehabilitation, jute bags filled with plant seeds and different substrates, in particular biochar and compost, are deposited on degraded land immediately before the onset of the rainy season in different shapes aiding water retention and windspeed reduction [11,12]. As the bags degrade, the substrates provide nutrients for plant growth and enhance ecosystem succession over the long term [13]. Thus, a single application of seeds and substrates can exert a lasting positive effect on initial ecosystem succession. Several ReviTec® research and demonstration sites were installed in the Northern regions of Cameroon. In the present study, we investigated, if substrate composition of the bags shaped soil aggregation and SOM storage over the longer term at the ReviTec® site on the campus of the University of Ngaoundéré, Adamaoua.

2. Materials and methods

2.1. Site description

The ReviTec® site in Ngaoundéré was installed in April 2012 on the premises of the University of Ngaoundéré at 7°25'21.1"N 13°32'22.5"E. Bags with different combinations of seeds and substrate were laid out on a sand-covered area in the form of islands. Each island consisted of four bags, two of which contained the same seeds and substrate. Established plant species on the site included *Indigofera hirsuta*, *Brachiaria brizantha*, and *Stylosanthes* sp. A sand-covered control area with no artificial seeding was installed next to the treated area. Of the five field replications, three were sampled for this work because two rows of islands were burnt and consistent conditions were not guaranteed anymore. For this study, the treatments "compost" (cp), "compost and biochar" (cpbc), "mineral control" (min) and the control area (ctr) were sampled (see table 1).

Table 1. Treatment composition on the ReviTec site in Ngaoundéré.

Treatment	Code	Composition (v/v)				Effect to be tested
		seeds	loamy sand	compost	biochar	
Control	ctr	-	-	-	-	control
Mineral matrix	min	yes	100 %	-	-	seeds in bag
Compost	cp	yes	70 %	30 %	-	compost
Compost and biochar	cpbc	yes	70 %	20 %	10 %	biochar

2.2. Field sampling

Topsoil samples were taken between November 25 and December 2, 2015 on the exact spots where the bags had been laid out. Aggregates were separated by dry sieving of bulk soil for two minutes, and rendered the following size classes: 1,000 – 2,000 µm (A), 400 – 1,000 µm (B), 200 – 400 µm (C), and < 200 µm (D). Each aggregate fraction was weighed to determine the gravimetric contribution to bulk soil. Bulk density, water holding capacity (WHC), and hydraulic conductivity were determined in the field (see appendix A1). For the ctr treatment, only one sample was taken and hence no standard errors were calculated.

2.3. Soil analysis

Gravimetric water content was determined by drying soil samples at 105 °C. For cation exchange capacity (CEC), samples were extracted with 10 mM $BaCl_2$ and elemental concentrations were measured using atomic absorption spectroscopy (AAS, Perkin Elmer, USA). Soil texture was measured using 35 % H_2O_2 digestion and 0.05 M $Na_4P_2O_7$ dispersion of the samples before determining the gravimetric contribution of every texture class to the entire sample ("pipette method"). Fractions > 63 µm were subsequently determined by wet sieving. pH was measured in a 1:5 (w/v) soil: 0.01 M $CaCl_2$ solution. SOM content was determined *via* loss on ignition by combustion at 450 °C. To assess microbial abundance, aggregate samples were fixed in a 4% formaldehyde solution, washed in PBS buffer and stored in a 1:1 (v/v) PBS:Ethanol solution. After sonication soil suspensions were filtered over a polycarbonate membrane (0.2 µm). Filter slices were impregnated with agarose and stained with 4',6-diamidin-2-phenylindol (DAPI) for cell counting. Bacterial cells were enumerated using an epifluorescence microscope (Carl Zeiss Axioskop 2, Carl Zeiss, Germany) with 350 nm excitation wavelength and a narrow band filter at an emission wavelength of 460 nm. Hyphal fragments were assessed using an excitation wavelength of 470 nm and a double excitation filter for best contrast. Bulk density, WHC, and pH were determined for bulk soil only, while soil texture, CEC, C and N contents, and microbial abundance were determined per aggregate size class.

2.4. Statistical analysis
All statistical analysis were performed in the R environment [18]. Differences between treatments and aggregate size classes were determined by one-way ANOVA. Conventional Tukey test at a significance level of 0.05 was used as a post-hoc test.

3. Results and discussion

3.1. Results

3.1.1. Bulk parameters. Bulk density was >1 g cm^{-3} for all of the investigated treatments, with lower values in the compost + biochar (cpbc) and compost (cp) treatments and the highest value in the control (ctr), although none of the differences were significant. WHC was between 20% and 30% based on soil dry weight (% dw), and was highest in the cp treatment and lowest in the ctr. Hydraulic conductivity was around $5 \cdot 10^{-4}$ ms^{-1} in the cp treatment and around $8 \cdot 10^{-4}$ ms^{-1} in the cpbc treatment. The min and control treatment displayed intermediate values between $6 \cdot 10^{-4}$ ms^{-1} and $7 \cdot 10^{-4}$ ms^{-1}. pH ($CaCl_2$) was around 5.7 without significant differences between the treatments (see table 2).

Table 2. Bulk parameters. Treatments: cpbc: compost + biochar, cp: compost, min: mineral matrix only, ctr: control area. Superscript letters indicate significant differences between treatments at $p < 0.05$. n.a: not assessed.

Parameter	Treatment	Mean	Standard Error
Bulk density [g cm^{-3}]	cpbc	0.999^{a}	0.039
	cp	1.041^{a}	0.077
	min	1.185^{a}	0.063
	ctr	1.373^{a}	0.087
Water holding capacity [% dw]	cpbc	26.701^{a}	1.668
	cp	31.238^{a}	1.281
	min	18.963^{a}	8.214
	ctr	n. a.	n. a.
Hydraulic conductivity [mm s^{-1}]	cpbc	0.82^{a}	0.08
	cp	0.492^{b}	0.074
	min	0.664^{ab}	0.021
	ctr	0.611	n. a.
pH(H_2O)	cpbc	5.703^{a}	0.067
	cp	5.743^{a}	0.381
	min	5.69^{a}	0.243
	ctr	n. a.	n. a.

3.1.2. Aggregate parameters. The general aggregation pattern was a left-skewed bell curve for every treatment, with highest gravimetric contributions by fraction B (~ 42 – 50 %) and lowest contributions by fraction D (~ 5 %). In cp, the contribution of fraction C was elevated compared to the other treatments, and fraction B contributed slightly less (see table 3). Texture did not differ between the treatments, but silt and clay content increased with decreasing aggregate size (see figure 1).

Table 3. Gravimetric contributions of aggregate fractions to bulk soil. Treatments: cpbc: compost + biochar; cp: compost; min: mineral matrix; ctr: control. Aggregate fractions: A: 1000 – 2000 µm; B: 400 – 1000 µm; C: 200 – 400 µm; D: < 200 µm.

Treatment	Aggregate fraction	Mean gravimetric contribution [% dw]
Cpbc	A	31.8
	B	50.4
	C	13.5
	D	4.3
Cp	A	33.4
	B	47.1
	C	14.8
	D	4.7
Min	A	32.1
	B	48.5
	C	14.7
	D	4.7
Ctr	A	39.0
	B	43.5
	C	13.1
	D	4.4

Residual water content (i.e. air-dry water content, rWC) was highest in cpbc and cp (up to 5 % dw), and due to large variation, no differences between aggregate fractions were found. In the min treatment, rWC was around 1.3 % dw, while in the ctr, rWC increased with decreasing aggregate size, from 0.5 % dw in A to ~ 3 % dw in D (see figure 2).

CEC was unchanged by treatment, but increased with decreasing aggregate size. Generally, CEC was very low with values between 1 and 5 $cmol_c$/kg. SOM content was between 1.7 % dw and 5.2 % dw in fractions A – C, with higher values in cpbc than in the other treatments. In fraction D, SOM content was around 13 % dw in cpbc, and between 8 % dw and 10 % dw in the other treatments. Base saturation was between 80 % and > 90 % in cpbc, and between 55 % and 75 % in the other treatments, without differences between aggregate fractions (see Table B1). Bacterial abundance was highest in cp ($7 – 8 \cdot 10^8$ cells/g dw), and lowest in the control ($5 – 6 \cdot 10^8$ cells (g dw)$^{-1}$). Cpbc and min treatments were similar at intermediate values. Differences between aggregate fractions were not significant (see Figure 3). Occurrence of fungal hyphae increased with decreasing aggregate size, while treatment had no significant effect (see figure 4).

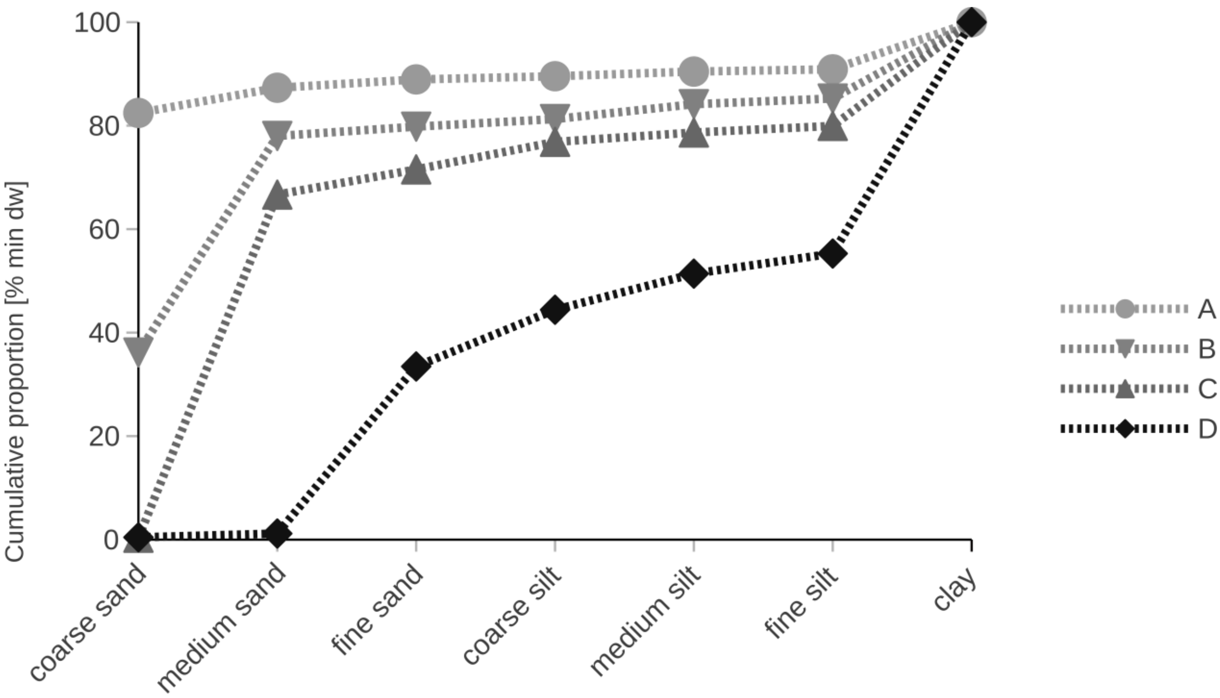

Figure 1. Texture in the aggregate fractions. Texture classes: coarse sand: 630 – 2000 µm; medium sand: 200 – 630 µm; fine sand: 63 – 200 µm; coarse silt: 20 – 63 µm; medium silt: 6.3 – 20 µm; fine silt: 2 – 6.3 µm; clay: < 2 µm. Aggregate fractions: A: 1000 – 2000 µm; B: 400 – 1000 µm; C: 200 – 400 µm; D: < 200 µm.

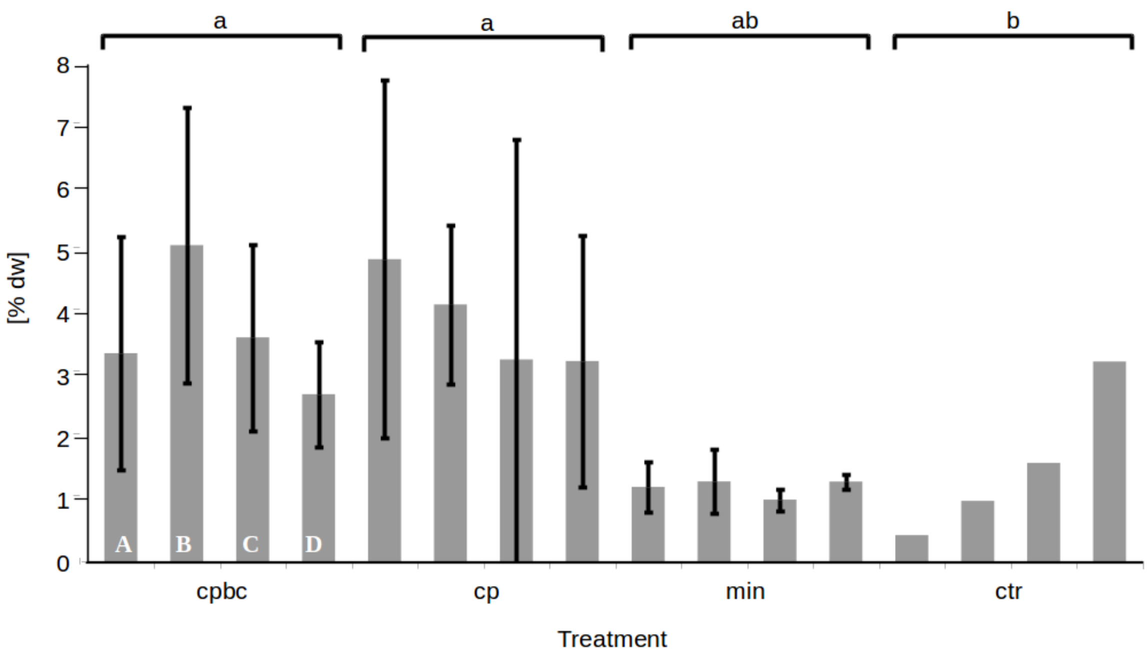

Figure 2. Residual water content of aggregate fractions. Treatments: cpbc: compost + biochar; cp: compost; min: mineral matrix; ctr: control. Aggregate fractions: A: 1000 – 2000 µm; B: 400 – 1000 µm; C: 200 – 400 µm; D: < 200 µm. Bars: standard error. Different minuscule letters indicate significant differences between treatments at a level of $p < 0.05$.

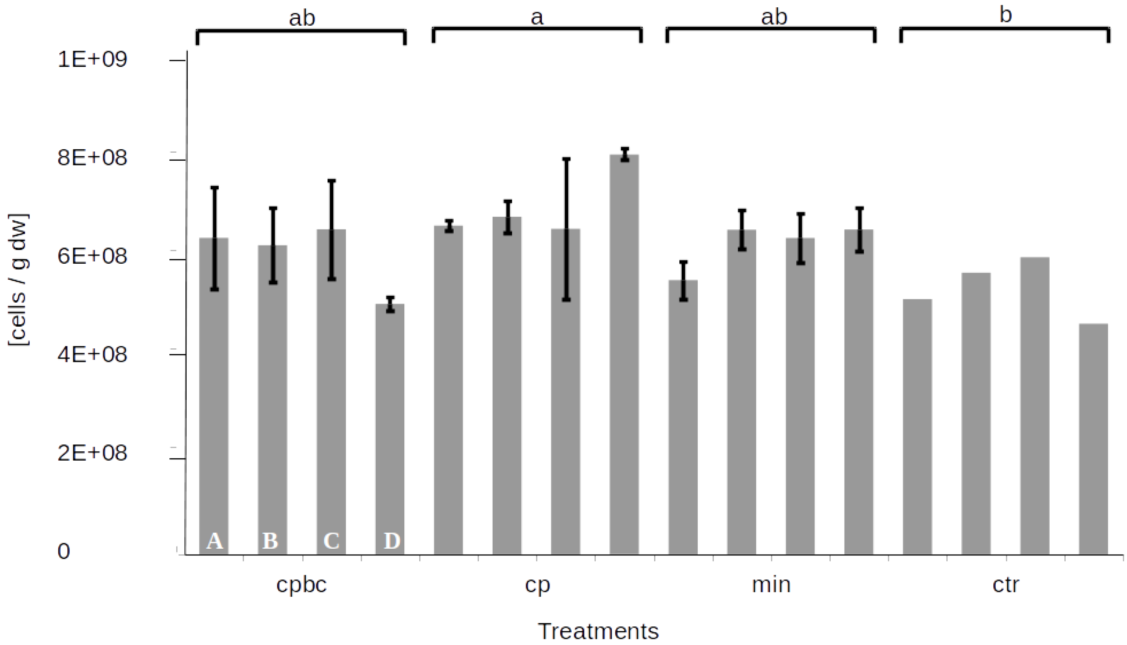

Figure 3. Bacterial abundance of aggregate fractions. Treatments: cpbc: compost + biochar; cp: compost; min: mineral matrix; ctr: control. Aggregate fractions: A: 1000 – 2000 µm; B: 400 – 1000 µm; C: 200 – 400 µm; D: < 200 µm. Bars: standard error. Different minuscule letters indicate significant differences between treatments at a level of $p < 0.05$.

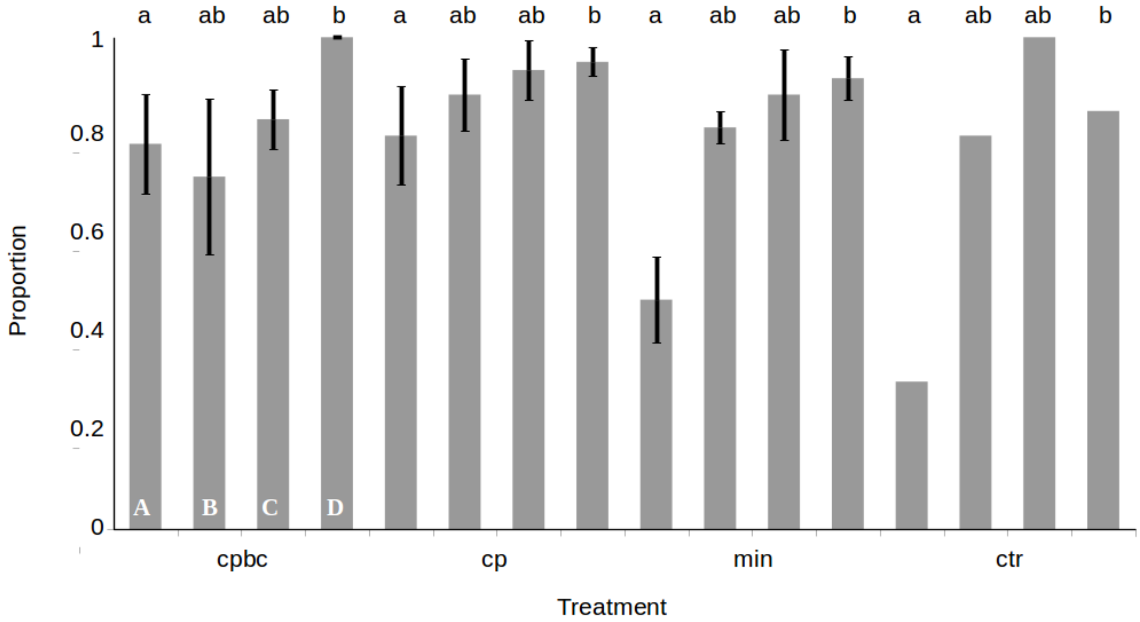

Figure 4. Fungal hyphae occurrence in aggregate fractions. Treatments: cpbc: compost + biochar; cp: compost; min: mineral matrix; ctr: control. Aggregate fractions: A: 1000 – 2000 µm; B: 400 – 1000 µm; C: 200 – 400 µm; D: < 200 µm. Bars: standard error. Different minuscule letters indicate significant differences between aggregate fractions across treatments at a level of $p < 0.05$.

3.1.3. Contribution of aggregate fractions to soil parameters. Mostly, contributions were related to gravimetric representation, meaning more represented size classes contributed more. Hence, larger fractions contributed most to all of the parameters. When the contribution was normalised to correct for gravimetric representation, aggregates < 200 µm contributed disproportionately more to chemical parameters. Vice versa, larger aggregate fractions contributed disproportionately less than would be assumed from their gravimetric representation. Larger aggregates contributed disproportionately more to rWC in the organic treatments. Bacterial abundance was the only parameter showing mostly proportionate contributions of all aggregate fractions (see table 4).

Table 4. Contribution of aggregate fractions to soil parameters. rWC: residual water content; SOM: soil organic matter; CEC: cation exchange capacity. Treatments: cpbc: compost + biochar; cp: compost; min: mineral matrix; ctr: control. Aggregate fractions: A: 1000 – 2000 µm; B: 400 – 1000 µm; C: 200 – 400 µm; D: < 200 µm.

Parameter	Treatment	Aggregate fraction	Contribution (%)	Normalised contribution	trend
rWC	cpbc	A	25.2	0.8	–
		B	60.2	1.2	+
		C	11.5	0.9	–
		D	2.7	0.6	–
	cp	A	38.6	1.2	+
		B	46.3	1.0	=
		C	11.4	0.8	–
		D	3.6	0.8	–
	min	A	31.6	1.0	=
		B	51.4	1.1	+
		C	12.0	0.8	–
		D	4.9	1.1	+
	ctr	A	17.6	0.5	–
		B	45.1	1.0	=
		C	22.1	1.7	++
		D	15.2	3.4	+++
SOM	cpbc	A	25.8	0.8	–
		B	45.3	0.9	–
		C	16.5	1.2	+
		D	12.4	2.9	+++
	cp	A	22.1	0.7	–
		B	49.1	1.0	=
		C	17.7	1.2	+
		D	11.2	2.4	++
	min	A	25.5	0.8	–

Parameter	Treatment	Aggregate fraction	Contribution (%)	Normalised contribution	trend
		B	37.7	0.8	–
		C	19.1	1.3	+
		D	17.6	3.8	++++
	ctr	A	21.0	0.5	–
		B	45.1	1.0	=
		C	17.3	1.3	+
		D	16.5	3.7	++++
CEC	cpbc	A	21.3	0.7	–
		B	49.0	1.0	=
		C	19.5	1.5	++
		D	10.1	2.3	++
	cp	A	24.4	0.7	–
		B	46.3	1.0	=
		C	19.4	1.3	+
		D	10.0	2.1	++
	min	A	25.6	0.8	–
		B	43.9	0.9	–
		C	19.2	1.3	+
		D	11.3	2.4	++
	ctr	A	27.3	0.7	–
		B	44.5	1.0	=
		C	18.3	1.4	+
		D	10.0	2.2	++
Bacterial abundance	cpbc	A	32.4	1.0	=
		B	50.0	1.0	=
		C	14.1	1.0	=
		D	3.5	0.8	–
	cp	A	32.7	1.0	=
		B	47.3	1.0	=
		C	14.3	1.0	=
		D	5.6	1.2	+
	min	A	28.6	0.9	–
		B	51.3	1.1	+
		C	15.2	1.0	=

Parameter	Treatment	Aggregate fraction	Contribution (%)	Normalised contribution	trend
		D	4.9	1.1	+
	ctr	A	36.7	0.9	–
		B	45.2	1.0	=
		C	14.4	1.1	+
		D	3.8	0.9	–

3.2. Discussion

3.2.1. Effect of the treatments. Only few changes between the treatments were detected in the bulk soil. The most prominent differences are the higher WHC and lower hydraulic conductivity in the compost treatment. While contradictory at a first glance, this can be explained by high casting activities of earthworms on these spots. The casts are very dense, harden quickly, and can retain high amounts of water, while at the same time cementing the soil and not allowing for water infiltration [14]. In clayey soils like at the site in Ngaoundéré, the arrangement of clay particles and the resulting pore geometry are important determinants of hydraulic features [15].

3.2.2. Changes in soil aggregation. Most prominent aggregate-specific changes occurred in the contribution of the aggregate fractions to the analysed parameters. Generally, the macroaggregate fractions contributed most, owing to their high gravimetric representation in bulk soil. Yet, microaggregates were four times richer in SOM than macroaggregates in all treatments, confirming their role in long-term SOM storage as often assessed by ^{13}C abundances [6]. Also, microaggregates contributed disproportionately more to chemical soil functions, confirming their role in long-term supply of nutrients. The large data variability in rWC of the organic treatments indicates higher heterogeneity in aggregate composition and perhaps also pore structure. In the compost treatment, medium-sized aggregates 200 – 400 µm contributed twice as much as in the other treatments, also owing to their greater representation in bulk soil. Soil under compost treatment was also enriched in bacteria, demonstrating high biological activity even far into the dry season. Liang *et al.* [16] found that organic amendments can protect bacterial colonies from drought, which is possible here as well. Fungi, on the other hand, appeared to be more robust and responded to aggregate size rather than to treatment. This is in line with our observations that clay content, CEC and SOM content both increase with decreasing aggregate size. Either fungi exploit the richer microaggregate fraction more than larger fractions, or their own biomass and mucilage contribute to high SOM contents, clay precipitation, and free exchange sites. Base saturation did not change with aggregate size, but was elevated only the combined treatment with biochar and compost. This indicates mineral depletion of the compost, mineral matrix, and control treatments, while stabilised and possibly inaccessible minerals were supplied by the biochar, probably due to a high ash content.

It appears that compost amendment had the most pronounced long-term effect on soil aggregation features, with particular enhancement of intermediate-sized aggregates and higher bacterial abundance even in the dry season. Little is known about the cascading effect of organic amendments on the soil food web, but results by Danra *et al.* [17] indicate that positive effects propagate to higher trophic levels and thus benefit the entire soil ecosystem.

4. Conclusions

We have demonstrated that biochar and compost can exert long-term positive effects on soil quality and soil aggregation in undisturbed soils. This makes them particularly efficient for rehabilitation efforts of degraded rangelands and other undisturbed forms of use. Compost-amended plots were attractive for earthworms whose casting activities changed soil hydraulic features. Bacteria were most attracted by the compost treatment, while fungi were encountered mostly in microaggregates of all treatment. In order for rehabilitation efforts to be successful, amendments must be specifically designed to enhance target parameters and target organismic groups, such as earthworms, bacteria, and fungi. We have furthermore confirmed the different functions of soil aggregate size fractions: Macroaggregates contribute most to soil water content during the dry season, while microaggregates resume important nutrient supply and SOM storage functions.

Appendices

Appendix A: Methodology for bulk density, water holding capacity, and hydraulic conductivity

Bulk density. 100 cm^3 pre-weighed steel cylinders were pushed into the topsoil to obtain soil cores. Cylinders with sample cores were weighed and the loss of volume from sampling artefacts was estimated. The resulting mass was further corrected for cylinder mass and bulk water content to obtain the bulk density [g/cm^3].
Water holding capacity. Soil cores from bulk density were transferred to pre-weighed funnels lined with wet filter paper. The samples were rinsed with demineralised water in excess to establish hydraulic conductivity and subsequently saturated and drained for at least 1 h. Drained weight was corrected for funnel and filter paper weight as well as bulk water content to obtain the water holding capacity [% dw].
Hydraulic conductivity. Sampling spots were watered twice 24 h and 5 h before sampling with 11 L of water. Plastic cylinders with a length of 23 cm and a volume of 1 L were pushed into the topsoil 3 cm. 1 L of water was poured into the cylinders quickly and time until all water had infiltrated was recorded. Water infiltration (m^3/s) was calculated by based on Darcy's law by multiplying the infiltration velocity (volume per time) with the length of infiltrated porous medium (3 cm) divided by the area of the infiltration ring (50 cm^2). To calculate hydraulic conductivity (m/s), a decreasing gradient (pressure head) per time was considered and the infiltration term was multiplied by the logarithm of initial height (23 cm) and final height of water in the infiltration ring (ln(23 cm 0.1 cm^{-1}) (see formula (1) with K = hydraulic conductivity [m/s], ρ = density of water [g cm^{-3}], g = gravitational acceleration constant [kg/m^3], κ = permeability [m^2] and μ = dynamic viscosity of water [Pa·s], see formula (A.1)).

$$K = \rho \cdot g \cdot \frac{\kappa}{\mu} \quad \text{(A.1)}$$

Table B1. Results for cation exchange capacity (CEC), soil organic matter content (SOM), and base saturation (BS). Means ± standard errors. Treatments: cpbc: compost + biochar; cp: compost; min: mineral matrix; ctr: control. Aggregate fractions: A: 1000 – 2000 µm; B: 400 – 1000 µm; C: 200 – 400 µm; D: < 200 µm. Superscript minuscule letters indicate significant differences between aggregate fractions at a level of $p < 0.05$. Superscript capital letters indicate significant differences between treatments at a level of $p < 0.05$.

Treatment	Aggregate fraction	CEC [$cmol_c$ kg^{-1}]	BS [%]	SOM [% dw]
Cpbc	A	1.29 ± 0.16^a	87.15 ± 2.95^A	3.60 ± 0.93^a
	B	1.87 ± 0.15^b	86.26 ± 3.95^A	4.00 ± 0.43^a
	C	2.77 ± 0.09^c	88.51 ± 4.62^A	5.42 ± 0.78^a
	D	4.50 ± 0.24^d	91.96 ± 2.96^A	12.77 ± 1.19^b
Cp	A	1.48 ± 0.18^a	66.79 ± 8.42^B	2.32 ± 0.30^a
	B	1.99 ± 0.09^b	65.82 ± 9.05^B	3.60 ± 0.30^a
	C	2.65 ± 0.26^c	63.51 ± 9.72^B	4.16 ± 0.77^a
	D	4.27 ± 0.35^d	75.84 ± 5.61^B	8.29 ± 0.25^b

Treatment	Aggregate fraction	CEC [$cmol_c$ kg^{-1}]	BS [%]	SOM [% dw]
Min	A	1.25 ± 0.11^a	65.55 ± 2.94^B	1.77 ± 0.26^a
	B	1.42 ± 0.06^b	58.55 ± 8.79^B	1.73 ± 0.28^a
	C	2.04 ± 0.15^c	54.41 ± 3.38^B	2.88 ± 0.36^a
	D	3.81 ± 0.15^d	55.87 ± 3.41^B	8.42 ± 1.00^b
Ctr	A	1.28	74.48	1.37
	B	1.88	68.77	2.63
	C	2.56	69.65	3.36
	D	4.13	67.65	9.44

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