Supplementary information document

Paper: Soil conditioner mixtures as an agricultural management alternative to mitigate drought impacts.

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Materials and methods

DNA was extracted from 3 soil washes, and 76 soil samples. Soil samples were obtained from the incubation experiment by collecting 2 mL of fresh soil which were then immediately stored at -20° C. All samples were extracted by means of a DNeasy PowerSoil Kit (Qiagen, Germany). The manufacturer's protocol was followed in the case of the soil samples, while in the case of the microbial wash, minor modifications were implemented. Soil samples were sublimed at a -20° C vacuum overnight to extract all moisture present at the moment of collection. Approximately 0.25 g of dry soil per sample were subjected to extraction. The microbial wash samples (2 ml) were centrifuged at 10000 g for 2 minutes. Excess water was removed, and the remaining solution was transferred into the PowerBead tube. After the final step, all three soil wash elusions were pooled in a single tube.

DNA concentration of each sample was determined by fluorometry in a Qubit 3 apparatus (Thermo Fisher Scientific, Germany) following the manufacturer's protocol. The ratio of bacterial to fungal DNA copy number ng^{-1} of DNA was estimated per sample by means of a quantitative PCR assay. Briefly, 5 µl of 20 % DNA template were added to 15 µl of master mix. Since the DNA concentration of the microbial wash pool was low, 5 µl of the undiluted extract were added to the master mix. The qPCR master mix contained 0.01 U $µl^{-1}$ of KAPA HiFi polymerase (Roche, Germany), 1x Mg buffer, 0.3 mM of dNTP`s mix, 0.5 µM of EvaGreen (Jena Bioscience, Germany), sterile H_2O and 0.25 µM of forward and reverse primers. To estimate the number of bacterial DNA copies, we employed primer pair 515F–926R (Parada et al., 2016; Quince et al., 2011). To estimate fungal copy number, we employed fITS7–ITS4 set (Ihrmark et al., 2012).

Calibration curves for qPCR assays derived from custom made standards for both groups of organisms. Standards were obtained by amplifying large fragments within the rDNA operon of bacteria or fungi. Briefly, 1 µl of a homogenized pool of soil DNA extracts (consisting of 1µl of each 23 samples) was used as template for these reactions. The bacterial standard (c 1600 bp) was obtained by running a 50 µl PCRs with primers 27f–1492r (Heuer et al., 1997). The fungal standard (c. 2900 bp) was obtained in a similar fashion, using primers NS31–LR5_F (Simon et al., 1992; Tedersoo et al., 2008). The concentration of the PCR products of these reactions were quantified fluorometrically as described before for the experimental samples. Standard DNA concentration, the target fragment size, an average molar mass bp^{-1} of 660, and the Avogadro`s constant, were then used to calculate the number of double stranded DNA copies present in each standard. Finally, a serial

dilution of each standard representing a concentration gradient of template DNA was prepared (standard ladder, 7 steps).

The plate layout for the qPCR assays was set as follows: The first two columns of each plate were loaded with 15 µl of master mix plus 5 µl of the corresponding standard ladder, or with 5 µl of sterile water in the case of negative controls. Four dilution steps of the standard ladder representing very high to low initial copy numbers, were replicated three times in each plate (n = 12). Negative controls and the lowest DNA concentration of the standard dilution were replicated only twice per plate. Finally, three technical replicates of each sample, including the microbial wash sample, were loaded (n = 72).

The qPCR program for bacteria it included a denaturation phase of 3 min at 95° C; 29 cycles of denaturation at 95° C for 20 s, annealing at 54°C for 30 s, elongation at 68° C for 30s; a final elongation step at 68° C for 5 minutes; and a scanning phase of a 65°–95° C for 15 min. For fungi the initial denaturation was at 98° C for 3 min, followed by 29 cycles of denaturation at 98° C for 20 s, annealing at 50° C for 20 s, elongation at 72° C for 30 s; a final elongation step at 72° C for 5 min and a scanning phase between 65°–95° C for 15 min. We ran all qPCR assays in a CFX96 cycler (Bio-Rad Laboratories, Germany).

At the end of each run the relative fluorescence unit (RFU) scores of each well in the assay were imported into program LinRegPCR (Ruijter et al., 2009) in order to estimate PCR efficiencies per sample. Only samples with efficiencies greater than 50% were included in the analysis. The PCR efficiencies served to calculate a corrected quantification cycle (Cq) for each standard and sample on the plate. The standard curve was based on the relation between the corrected Cq scores of each standard dilution and their copy number estimate (Ruijter et al., 2021). A power function was fitted to this relationship (Fig. S1A, B). The equation from this fit was used to estimate the copy number of bacterial and fungal DNA present on each sample, based on their corrected Cq scores.

All the data produced in this assay and used for calculations can be found in https://doi.org/10.5281/zenodo.13311518

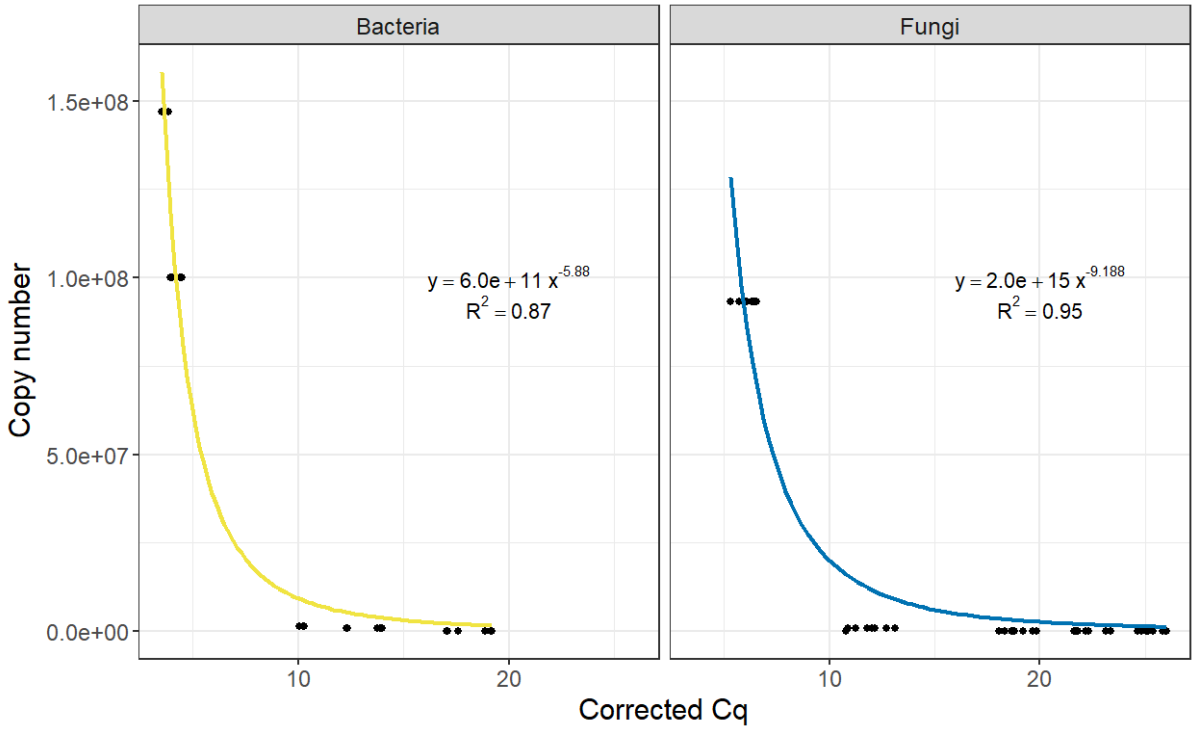

Figure S1. qPCR for calibration curves for A. bacteria, and B. fungi. The standard range was 1465.89–1.47E+08 for bacteria, whereas for fungi was 93.1–9.31E+07 copies. The equations corresponding to the power functions depicted in the plots were used to estimate the copy number based on the corrected Cq of each sample in the assay.

Figure S2. Effects of the addition of individual amendments on the soil processes measured. The effect of each amendment type is measured in relation to non-amended soils (i.e. control). Faded circles represent individual samples, while the distribution to the right represents a density function. Model coefficients are presented in Table S3.

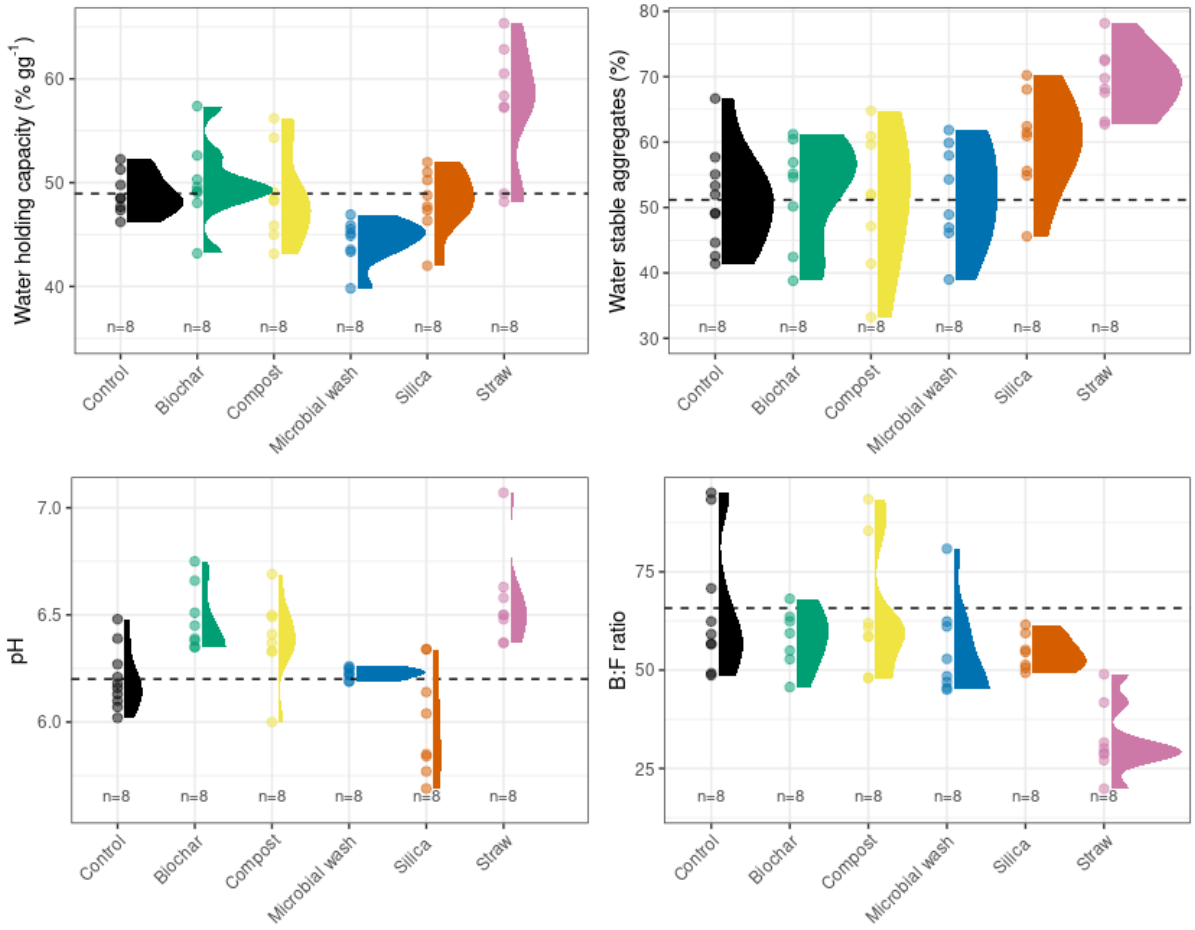

Figure S3. Conditioner richness compared against non-amended soils and a single conditioner at three times the individual dosage used in any of the mixtures (Biochar 3x). Each panel represents one of the variables monitored in this study. A label that describes the amendments contained on each random 3-way mixture was plotted next to the points representing a sample in this category. B=biochar, C=compost, M=microbial wash, SL=amorphous silica, SW=wheat straw.

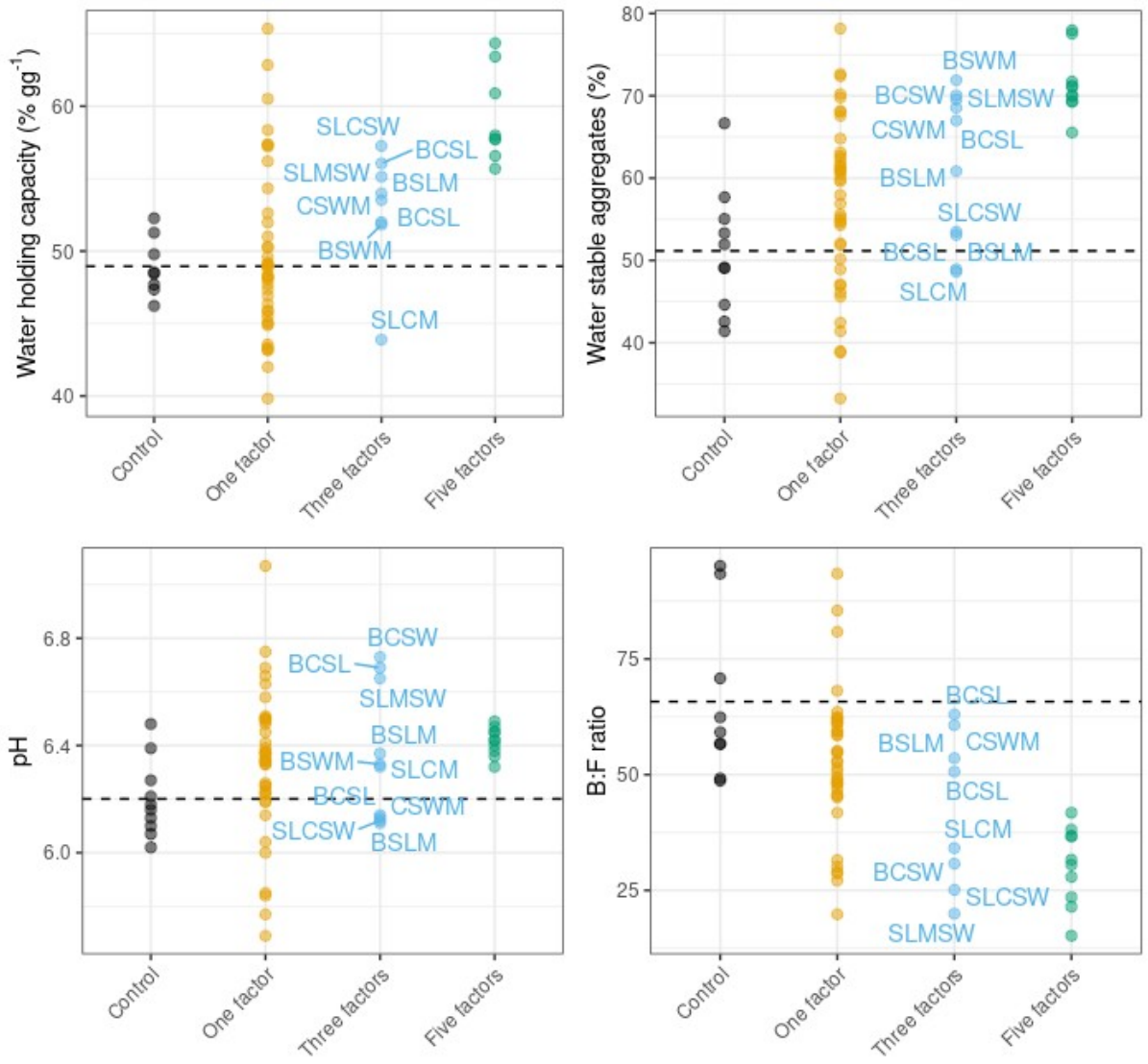

Table S1. Coefficients of all the models in which the number of factors is compared to the non-amended soils (Control or Intercept), and all the individual factors combined. The first two variables were fit with beta regressions while pH and B:F ratio were fitted with linear models. Consequently, the first two models contain coefficients for a precision estimate (phi).

Response	Factor	Estimate	Std. Error	Wald Z	p
mWHC	Intercept	-0.042	0.070	-0.599	0.549
	Nfactors1	0.032	0.077	0.411	0.681
	Nfactors3	0.159	0.099	1.609	0.108
	Nfactors5	0.416	0.100	4.167	0.000
	phi	101.044	17.775	5.685	0.000
WSA	Intercept	0.046	0.115	0.405	0.685
	Nfactors1	0.236	0.129	1.839	0.066
	Nfactors3	0.408	0.164	2.482	0.013
	Nfactors5	0.839	0.170	4.934	0.000
	phi	29.344	4.881	6.012	0.000
pH	Intercept	6.201	0.074	84.045	0.000
	Nfactors1	0.131	0.082	1.588	0.117
	Nfactors3	0.158	0.104	1.514	0.135
	Nfactors5	0.215	0.104	2.061	0.043
B:F ratio	Intercept	65.781	5.015	13.116	0.000
	Nfactors1	-13.044	5.578	-2.339	0.023
	Nfactors3	-23.537	7.311	-3.219	0.002
	Nfactors5	-35.419	6.913	-5.123	0.000

Table S2 Model coefficients of all the models in which the number of factors is compared to the non-amended soils (Control or Intercept) and one factor at three times the concentration used on any particular mixture (Biochar, triple dosage). The first two variables were fit with beta regressions and the third (pH) is a liner model. Consequently, the first two models contain precision estimates (phi).

Response	Factor	Estimate	Std. Error	Wald Z	p
mWHC	Intercept	-0.042	0.042	-1.010	0.312
	Nfactors1	-0.015	0.059	-0.257	0.797
	Nfactors3	0.160	0.059	2.713	0.007
	Nfactors5	0.419	0.060	7.026	0.000
	phi	285.521	71.256	4.007	0.000
WSA	Intercept	0.047	0.098	0.477	0.634
	Nfactors1	0.179	0.140	1.280	0.200
	Nfactors3	0.412	0.141	2.919	0.004
	Nfactors5	0.848	0.146	5.800	0.000
	phi	40.246	8.895	4.524	0.000
pH	Intercept	6.201	0.049	125.505	0.000
	Nfactors1	0.456	0.070	6.526	0.000
	Nfactors3	0.158	0.070	2.261	0.030
	Nfactors5	0.215	0.070	3.077	0.004
B:F ratio	Intercept	65.781	4.390	14.984	0.000
	Nfactors1	-10.713	5.377	-1.992	0.053
	Nfactors3	-23.537	6.399	-3.678	0.001
	Nfactors5	-35.419	6.051	-5.853	0.000

Table S3 Model coefficients of the four models in which each factor added individually is compared to the non-amended soils (Control or Intercept). The first two variables were fit with beta regressions whereas pH and B:F ratio were fitted with linear models. Consequently, the first two models contain precision estimates (phi).

Response	Factor	Estimate	Std. Error	Wald Z	p
mWHC	Intercept	-0.042	0.052	-0.808	0.419
	Biochar	0.039	0.074	0.531	0.596
	Compost	-0.007	0.074	-0.095	0.924
	Microbial wash	-0.183	0.074	-2.474	0.013
	Silica	-0.031	0.074	-0.419	0.675
	Straw	0.340	0.074	4.589	0.000
	phi	183.186	37.291	4.912	0.000
WSA	Intercept	0.047	0.096	0.491	0.623
	Biochar	0.050	0.144	0.351	0.726
	Compost	0.006	0.144	0.045	0.964
	Microbial wash	0.027	0.144	0.186	0.852
	Silica	0.353	0.145	2.431	0.015
	Straw	0.757	0.150	5.052	0.000
	phi	42.689	8.442	5.057	0.000
pH	Intercept	6.201	0.057	109.059	0.000
	Biochar	0.279	0.085	3.271	0.002
	Compost	0.189	0.085	2.216	0.032
	Microbial wash	0.025	0.085	0.296	0.769
	Silica	-0.200	0.085	-2.342	0.024
	Straw	0.362	0.085	4.239	0.000
B:F ratio	Intercept	65.781	4.182	15.731	0.000
	Biochar	-7.643	6.322	-1.209	0.234
	Compost	-1.419	6.096	-0.233	0.817
	Microbial wash	-10.390	6.096	-1.704	0.096
	Silica	-11.210	6.322	-1.773	0.084
	Straw	-33.656	6.096	-5.521	0.000

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