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Short-Term Effect of Biochar on Microbial Biomass, Respiration and Enzymatic Activities in Wastewater Irrigated Soils in Urban Agroecosystems of the West African Savannah

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Abstract: Irrigated urban agriculture (UA) supports the economy and health of urban inhabitants in low-income countries. This system is often characterized by high nutrient inputs and mostly utilizes wastewater for irrigation. Biochar has been proposed to increase crop yields and improve soil properties. In this study, we assessed the transient effect of rice husk biochar (20 t ha^{-1}) and/or fertilizer (NPK: 15-15-15) on microbial respiration, microbial biomass carbon and enzyme activities of irrigated (wastewater and tap water) soil from an UA field experiment in the Guinea savannah zones of Ghana. Our results showed an increase by up to 123% in soil organic carbon (SOC) after a year of biochar application, while hot water extractable carbon (HWEC) was increased by only 11 to 26% and microbial biomass carbon (MBC) by 34%. Basal respiration was significantly increased in mineral fertilized soil by up to 46% but decreased by 12–45% under wastewater irrigation. Overall, the metabolic quotient (qCO_2) indicated less stress for the microbial community and increased carbon use efficiency with biochar application and wastewater irrigation. Total enzymes activity was increased under wastewater irrigation and biochar treated soils exhibit a more diverse composition of C-cycling enzymes and a higher activity of aminopeptidases. Biochar and wastewater showed positive effects on biological soil properties and contributed to soil fertility. Our results suggest beneficial effects of biochar on non-biochar SOC stocks in the long term.

Keywords: urban agriculture; wastewater; biochar; microbial activities; fertilization

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1. Introduction

Irrigated urban agriculture (UA) is a common phenomenon in African cities that contributes to 60–100% of the fresh vegetable supply [1]. It makes use of free spaces, provides income for farmer households and contributes to more diverse diets [2]. It is often characterized by excessive use of mineral or organic fertilizer, intensive continuous cropping cycles without fallow periods, production of cash crops and irrigation with untreated sewage water [3]. Soil fertility in these systems is often highly weathered and very poor. For instance, Häring et al. [4] reported very low soil organic carbon, nitrogen, cation exchange capacities and pH values for UA soil in Tamale, Ghana. Nutrient leaching in UA systems can be very high due to high nutrient inputs and poor soil properties (low activity clay and low cation exchange capacity) [5,6]. However, little is known about soil biological properties and its contribution to the nutrient cycle in these agroecosystems.

Soil biological activities are an important part of soil quality since microorganisms and their associated enzymes in soil are responsible for the breakdown of organic matter

and release of nutrients [7]. Especially, irrigation with wastewater can have a strong impact on soil microbial activities and abundance due to the input of carbon or mineral and organically bound nutrients [8,9]. Irrigation with raw or treated wastewater has been shown to have effects on soil enzyme activities and thus turnover of nutrient and organic carbon [10,11]. In the West African savannah soils, organic matter content is inherently low due to climatic constraints and poor soil formation [6]. Application of biochar in such a highly weathered soil could potentially improve its physicochemical and biological properties. A recent meta-analysis of 109 studies by Jeffery et al. [12] revealed a 25% average increase in yield through biochar application in tropical climates. This effect was mainly explained by the liming and fertilization effect of ash content of biochar [12]. Other authors suggest that biochar may increase microbial activity, provide habitat for microorganisms and hence alter microbial mediated processes in soil [13]. Especially, soil enzyme activities are affected by biochar. For instance, Paz-Ferreiro et al. [14] found an increase in soil enzyme activities driving important processes in soil when biochar was added to tropical soil. However, Lammirato et al. [15] reported a decrease in β-glucosidase activities with biochar application. Furthermore, biochar carbon has shown to be very recalcitrant in soil with turnover times of more than a millennium [16] thus potentially providing a long-term improvement of soils.

We hypothesized that biochar amendment would significantly increase microbial abundance and activities in wastewater irrigated soil and therefore contribute to its fertility level. The objectives of the study were to determine the effect of biochar amendment in combination with wastewater irrigation on: (1) soil enzymatic activities for C, N and P hydrolysis; (2) substrate induced microbial respiration and biomass for carbon turnover; and (3) total nutrient (C, N, P) and hot water extractable carbon.

2. Materials and Methods

2.1. Experimental Design and Site Description

A field experiment was established in 2014 to test the influence of biochar and/or fertilizer on soil properties and yields under fresh and wastewater irrigation. The experiment was conducted in Tamale, northern region of Ghana, West Africa. The site is located in the Guinea savannah zone and has a semi-arid climate with a monomodal rainfall pattern from May to mid-October. The mean annual precipitation is 1090 mm and the daily mean temperature is 27.9 °C. The soil at the experimental site is a Petroplinthic Cambisol [17] with 45.7% sand, 48.40% silt and 5.90% clay. Effective cation exchange capacity (CEC) of the initial soil measured 33 mmolc kg^{-1} while total nitrogen (N) and soil organic carbon (SOC) was 0.4 and 4.1 g kg^{-1} respectively (Table 1). Soil treatments in the field experiment comprised of a control (with no amendments), biochar treatment (20 t ha^{-1}) incorporated and thoroughly tilled manually within the top 20 cm of the soil, fertilized treatment according to the normal agricultural practices (NAP) of the local farmers and a treatment with biochar and NAP. Multiple crop cycles were conducted on the field between May 2014 and April 2015. The crop cycles consisted of maize (*Zea mays* L. grown only up to the vegetative stage), lettuce (*Lactuca sativa* L.), cabbage (*Brassica oleracea* var. capitata L.) amaranth (*Amaranthus cruentus* L.) and jute mallow (*Corchorus spp*). At each cropping cycle, all plots were cultivated with the same crop. Every treatment was replicated four times and either irrigated with clean tap water or with untreated wastewater. The wastewater contained a relatively higher amount of NH_4-N (35.54 mgL^{-1}), PO_4-P (8.13 mgL^{-1}) and electrical conductivity (EC) 546.31 µS cm^{-1} in comparison with tap water which had 0.04 mgL^{-1}, 0.05 mgL^{-1} and 97.51 µS cm^{-1} of NH_4-N, PO_4-P and EC respectively (Table 1). The fertilizer used in the NAP treatment was a commercial NPK 15-15-15 blend and applied by broadcasting. During the first year of the experiment, the NAP treatments received 228.7 kg N ha^{-1}, 97.7 kg P ha^{-1} and 141.8 kg K ha^{-1} in the form of NPK (15-15-15) fertilizer. The biochar was produced from rice husks, which is an abundant organic waste in Ghana [18]. Briefly, the rice husks were heated in a custom pyrolysis oven under limited oxygen conditions to about 550 °C with an average resident time of 43 h. The produced

biochar had a particle size less than 2 mm and therefore was applied without griding. The organic C content of the biochar reached an average of 42.4% and low N-contents of 0.6% (Table 1). In April 2015, thus one year after the field's establishment, soil samples were taken by gaining a composite sample from six randomly distributed points on each plot at a depth of 20 cm. After sampling, the soils were air dried, sieved to 2 mm and shipped to Germany for further analysis of soil biological parameters.

Table 1. Initial properties of biochar and soil (A) and average concentration (n = 20) of nutrients and trace elements (B) in irrigation water inputs (adopted from [4,19]). ND represents not-detected.

A.	Initial Properties			B.	Irrigation Water Inputs		
Parameter	**Unit**	**Soil**	**Biochar**	**Parameter**	**Unit**	**Wastewater**	**Tap Water**
Sand	%	45.7	–	NO_3-N	mgL^{-1}	0.16	0.27
Silt	%	48.40	–	NH_4-N	mgL^{-1}	35.54	0.04
Clay	%	5.90	–	PO_4-P	mgL^{-1}	8.13	0.05
CEC	$mmol_c\ kg^{-1}$	36.10		pH	-	7.37	7.56
pH		5.10	9.1	EC	$\mu S\ cm^{-1}$	546.31	97.51
SOC	%	0.41		K	mgL^{-1}	4.46	1.16
Bulk density	$g\ cm^{-3}$	1.42		Al	mgL^{-1}	0.055	0.05
Carbon	%	0.40	42.4	Fe	mgL^{-1}	0.47	0.68
Nitrogen	%	0.04	0.6	Zn	mgL^{-1}	0.01	0.05
Avail. phosphorus	$mg\ kg^{-1}$	7.70	nd	Cu	mgL^{-1}	0.01	0.01
Total phosphorus	$mg\ kg^{-1}$	110.9	861.3	Mn	mgL^{-1}	0.33	0.03
Potassium	$mg\ kg^{-1}$	38.9	977.1	Pb	mgL^{-1}	1.82	nd
BET	$m^2\ g^{-1}$	–	62.9	Ni	mgL^{-1}	0.1	nd
Volatile matter	%	–	23.2	Cd	mgL^{-1}	0.01	0.01
Ash content	%	–	45.2	As	mgL^{-1}	0.05	0.02
H/C *(molar ratio)*		–	0.05	Ba	mgL^{-1}	0.05	0.07
O/C *(molar ratio)*		–	0.27	Mo	mgL^{-1}	0.03	0.07

2.2. Analysis of Soil Chemical Parameters

The total carbon and nitrogen contents of the samples were determined by using a C/N-analyzer (Vario max cube, Elementar Analysesysteme GmbH, Hanau, Germany). The pH was measured by mixing soils with 0.1 M $CaCl_2$ at a 1:5 soil: solution ratio (vol.: vol.). Readings were taken with a pH meter equipped with a gel electrode (Sentix 41, Wissenschaftlich-Technische Werkstätten (WTW) GmbH, Weilheim, Germany). Hot water extractable carbon (HWEC) was measured with a method adapted from [20]. Briefly, 2 g pre-incubated soil was mixed with 20 mL deionized water in centrifuge tubes. After 16 h in an 80 °C water bath the tubes were centrifuged and carefully decanted. The supernatants were filtered (0.45 µm membrane) and then analyzed with a total organic carbon (TOC)-analyzer (Dimatoc 2000, Dimatec, Essen, Germany).

2.3. Assessment of Soil Respiration, Microbial Biomass and Enzyme Activity

Prior to the measurement of soil biological parameters, the samples were rewetted to 50% water holding capacities (WHC) and pre-incubated for seven days at 25 °C. Basal and substrate-induced respiration were measured with the Microresp method developed by [21]. Briefly, a pre-incubated sample (0.3 g) of soil was placed into a well in a 96 deep-well microtiter plate and pure water (for basal respiration) or substrate (glucose, alanine or citric acid at 30 mg C g^{-1} soil) was added to reach 60% WHC. Afterward, the plate was covered and sealed with another plate filled with agar containing NaOH and an indicator dye (cresol red). The evolved CO_2 was captured in the agar and causes a color change. After six hours of incubation at 25 °C, the color change was measured with a microplate reader (Infinite F200, TECAN Instruments, Crailsheim, Germany) at 590 nm.

Microbial biomass carbon (MBC) was determined with the chloroform fumigation extraction method according to [22]. An amount of 10 g pre-incubated soil samples

was exposed to chloroform vapor for 24 h in a desiccator and extracted afterward with 40 mL 0.5 M K_2SO_4 by horizontal shaking (200 rev.min^{-1}). TOC of the extracts was measured with a TOC analyzer (Dimatoc 2000, Dimatec, Essen, Germany). In addition, an unfumigated sample (10 g) was extracted the same way and microbial biomass carbon was then calculated as the difference in TOC of fumigated and unfumigated sample by using a conversion factor of 0.45 [23].

Dehydrogenase activity was determined by measuring the reduction of 2-p-iodophenyl-3-p-nitrophenyl-5-phenyl-tetrazoliumchloride (INT) to iodonitro-tetrazoliumformazan (INTF) after 24 h [24]. One-gram dry weight (DW) of field-moist soil was mixed with 50 µl glucose solution (1%) and 1 mL INT solution (0.4%) and in-cubated for 24 h in the dark at 23 °C. Then, 10 mL methanol was added to stop the re-action and after filtration, the extinction of the extract was measured at 485 nm and INTF content was calculated using a calibration curve.

Extracellular enzyme activities of the C, N, P, and S cycle were measured after [25] with fluorescent substrates. The following extracellular enzyme activities were measured for C-cycling enzymes: α-glucosidase (α-glu), β-xylosidase (β-xyl), β-glucosidase (β-glu), N-acetyl-glucosidase (N-acet), and β-cellobiosidase (β-cello) were measured with methy-lumbelliferyl (MUF)-labeled substrates. Additionally, acid phosphatase (pho) involved in the P cycle was measured with MUF-labeled substrates. Activities of enzymes from the N cycle, such as leucine-aminopeptidase (leu), tyro-sine-aminopeptidase (tyr), and arginine-aminopeptidase (arg) were determined with amido-methylcoumarin (AMC)-labeled substrates. The respective substrate (1 mM) was dissolved in dimethyl sulfoxide and diluted with sterile water and 0.1 M 2-N-morpholinoethanesulfonic acid (MES) buffer for MUF substrates or 0.05 M tris(hydroxymethyl)aminomethane (TRIZMA) buffer for AMC substrates. A soil sus-pension was produced by mixing 1 g of soil with 50 mL sterile water and treated with an ultrasound probe at 150 W to release the enzymes from the soil particles. Soil sus-pension and substrates in the respective buffer solutions were pipetted into microtiter plates and incubated for 10 min at 30 °C. Subsequently, enzyme activities were meas-ured by detecting florescence of released enzymatic products every 30 min within a 3 h period with a multiplate reader (Infinite F200, TECAN Instruments, Crailsheim, Ger-many) at 360 nm excitation and emissions at 465 nm. The enzyme activities were cal-culated from the slopes of the resulting utilization curves.

2.4. Statistical Analysis

Significant differences of means were assessed by using multifactorial analysis of variance (MANOVA) taking biochar, fertilizer, water quality and block effects as factors. The block effects were calculated to consider the field heterogeneity. p values <0.05 are considered to be significantly different. Data were checked for normal distribution with the Shapiro-Wilk test. Where necessary, data were transformed to achieve normal distribution. Principal component analysis (PCA) was used for the reduction of parameters to two main factors. Varimax rotated factor loadings are given in Figure 4. MANOVA was done using R [26] and PCA was conducted with SPSS Statistics 22 (IBM, Armonk, NY, USA).

3. Results

3.1. Treatment Effects on Soil Chemical Parameters

The addition of biochar caused an increase in SOC by up to 123% in the unfertilized treatments and 86% in the NAP which included NPK addition. Hot water extractable carbon (HWEC), an indicator of microbial based SOC was increased in biochar amended plots by 26% in the unfertilized soils with both irrigation water qualities. Similar significant increase in HWEC was observed in NAP treated soil where NPK fertilization was applied (Table 2). Biochar amendment significantly raised the C/N ratio to an average of 16.1 from 8.1 in the unamended control but was reduced to 13.27 in the combined biochar and NAP treated plots. Fertilizer addition decreased pH of the soil by 0.7 units but was elevated up to 0.24 in biochar amended plots. Total P in soil was significantly increased between

45–50% with fertilizer use in the NAP treatments compared to the control (Table 2). In the biochar treatments, no significant effect on total P and N in soil was found. Compared to tap water irrigated soil, wastewater irrigation led to a slight increase in the amount of N (10%) and P (12%), but was only significant with regards to concentration of HWEC (Table 2).

Table 2. Chemical properties of soil under two different irrigation water treatments, statistically significant effects and interactions are indicated by *p*-values ($p < 0.05$) of MANOVA. Means ± one standard deviation, $n = 4$. Transformation applied after Shapiro Wilk test are indicated for each parameter. BC, Fert and Watqu in the table represent biochar, fertilizer and water quality respectively.

Water Quality	Soil Treatment	C [g kg^{-1}] ±SD	N [g kg^{-1}] ±SD	C: N -	*p* [g kg^{-1}] ±SD	pH [-] ±SD	HWEC [g kg^{-1}] ±SD
Tap water irrigation	Control	3.5 ± 0.7	0.4 ± 0.1	7.94 ± 0.57	0.14 ± 0.01	5.2 ± 0.24	166.49 ± 10.9
	Biochar	6.9 ± 0.8	0.5 ± 0.1	15.81 ± 2.27	0.17 ± 0.02	5.23 ± 0.32	210.2 ± 7.95
	NAP	4.3 ± 0.4	0.5 ± 0.0	8.94 ± 0.64	0.2 ± 0.01	4.51 ± 0.16	191.69 ± 4.23
	NAP/Biochar	8 ± 2	0.6 ± 0.0	12.92 ± 1.21	0.2 ± 0.01	4.54 ± 0.18	212.37 ± 16.75
Wastewater irrigation	Control	3.5 ± 1	0.5 ± 0.1	8.26 ± 0.64	0.16 ± 0.02	5.04 ± 0.19	173.08 ± 7.16
	Biochar	7.8 ± 1.2	0.5 ± 0.1	16.39 ± 1.67	0.19 ± 0.02	5.25 ± 0.25	218.1 ± 2.19
	NAP	4.5 ± 0.3	0.5± 0.1	9.33 ± 1.02	0.23 ± 0.02	4.67 ± 0.12	197.94 ± 8.44
	NAP/Biochar	8.3 ± 0.9	0.5± 0.0	13.62 ± 1.34	0.21 ± 0.01	4.58 ± 0.07	223.43 ± 4.0
Transformation		log	log	log			
p values (MANOVA)							
Block							
Biochar		<0.05		<0.05			<0.05
Fertilizer			<0.05		<0.05	<0.05	<0.05
Water Quality							<0.05
Sig. Interactions ($p \leq 0.05$)				BC:Fert	BC:Fert BC:Watqu		BC:Fert

3.2. Substrate Induced Microbial Respiration

Wastewater irrigation shows a mean basal respiration of 0.57 CO_2-C g^{-1} and leads to significant lower values over all treatments than tap water irrigation where 0.65 µg CO_2-C g^{-1} mean values were detected. Biochar incorporation in our study did not affect basal respiration neither for tap nor wastewater irrigated samples. Amongst the substrates, citric acid had the highest mean respiration rate but was not influenced by either soil treatment or water quality (Figure 1). Fertilizer addition in NAP plots reduced the microbial respiration under added glucose (41%) and alanine (46%).

3.3. Microbial Biomass Carbon and Metabolic Quotients

Microbial biomass carbon was 51.05 mg kg^{-1} in soil irrigated with wastewater compared to a mean of 46.26 mg kg^{-1} through tap water irrigation. Soils containing biochar had a relative higher mean value for microbial biomass carbon with 54.9 mg kg^{-1} in the sole biochar treatments and 57.49 mg kg^{-1} in the soil treated with the combination of biochar and NAP compared to the control (41.72 mg kg^{-1}) and NAP soils (41.34 mg kg^{-1}). Metabolic quotient (qCO_2) ranged between 0.01 to 0.02 µg CO_2-C (mg microbial C) $^{-1}$ h^{-1} and was 12–45% lower in soil samples from wastewater irrigated plots (Figure 2). Biochar also had a decreasing effect on the qCO_2 whereas, in the NAP treatments, the qCO_2 values were increased under both irrigation water qualities.

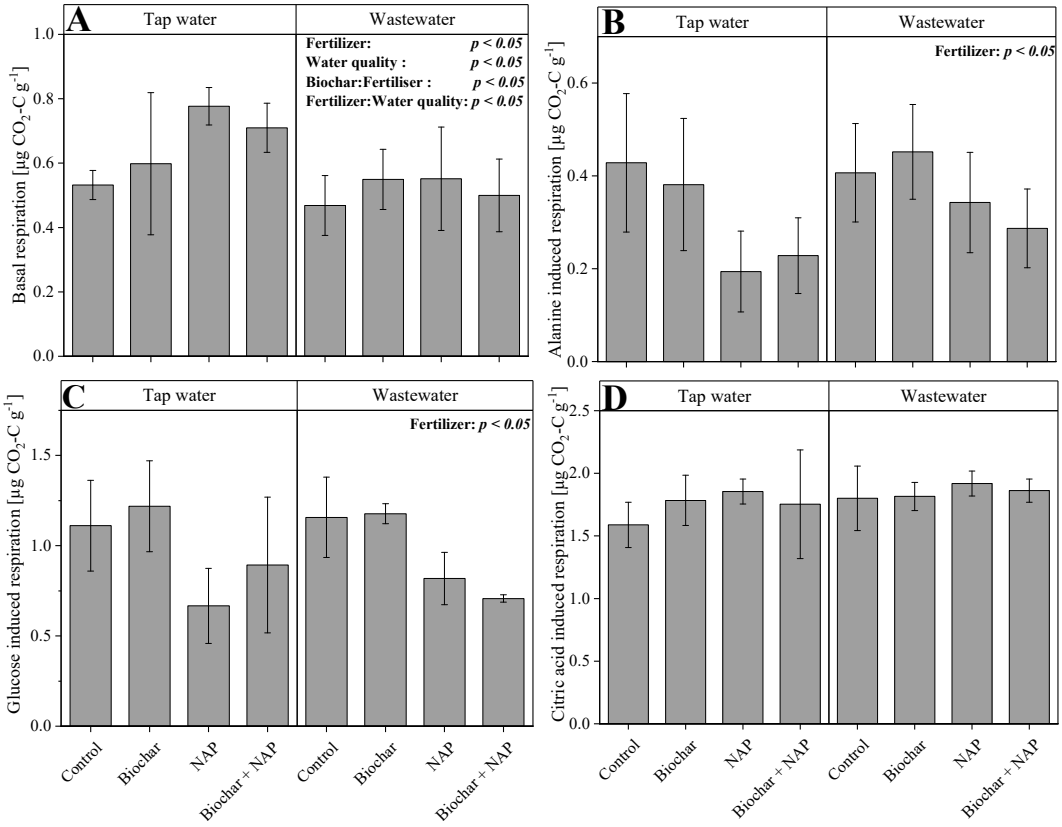


Figure 1. Average basal (**A**), alanine respiration (**B**), glucose (**C**) and citric acid (**D**) induced microbial respiration of soil. Error bars represent standard deviation, $n = 4$. Basal respiration was squared to obtain normal distribution after the Shapiro Wilk test. Statistically significant effects of treatments (biochar, fertilizer, water quality and its interactions) from MANOVA ($p < 0.05$) are noted in the graphs, non-significant effects are not indicated.

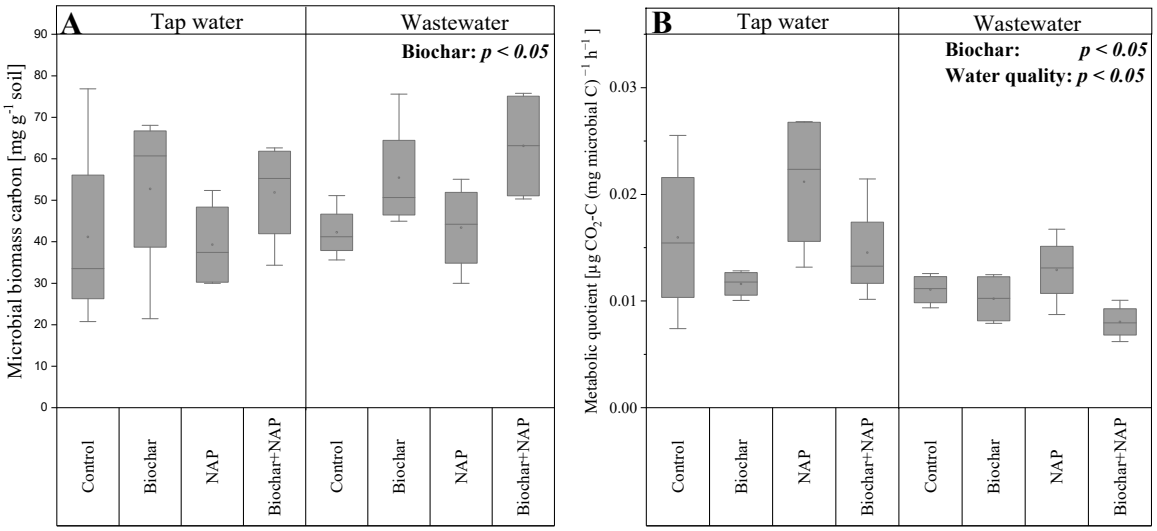


Figure 2. Microbial biomass carbon (**A**) and metabolic quotient (**B**) of soil. Whiskers on boxplots represent 95% and 25% percentile. Statistically significant effects of the treatments (biochar, fertilizer, water quality and interactions) from MANOVA ($p < 0.05$) are indicated in the graphs.

3.4. Enzyme Activities

The extracellular enzymatic activities determined by the fluorimetric assay showed the most sensitive reaction to the different irrigation water qualities. This is clearly visible in Figure 3, where the summed enzyme activities for the three nutrient cycle groups are shown. In the wastewater irrigated soils, the overall enzyme activities (7874.93 µmol product g^{-1} soil h^{-1}) were approximately 2 to 3 times higher than in tap water irrigated soils (2920.71 µmol product g^{-1} soil h^{-1}) which is largely due to increases in N-cycle enzymes (Figure 3), especially of arginine-aminopeptidase (Table 3). Under tap water irrigation, NAP greatly suppressed the activity of the C-cycle enzymes to about 22% of the control and biochar treatments. Interestingly, biochar alone did not affect the C-cycle enzymes while greatly increasing the activity of N-cycle enzymes under both irrigation water qualities. The only stimulation of C-cycle enzymes occurred with biochar + NAP under wastewater irrigation where activities were about 4-fold higher than in the control (Figure 3), largely due to the extreme increase in β-glucosidase activity (Table 3).

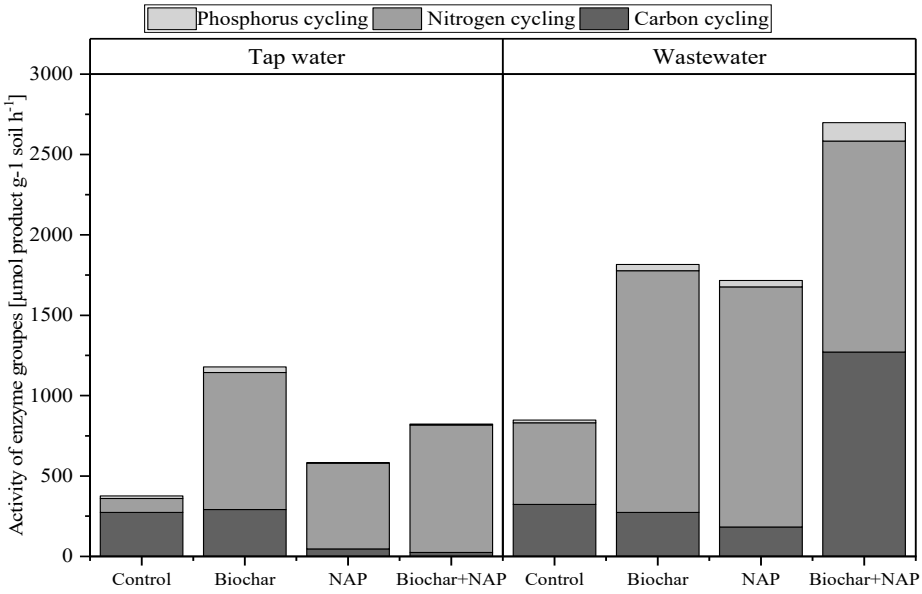


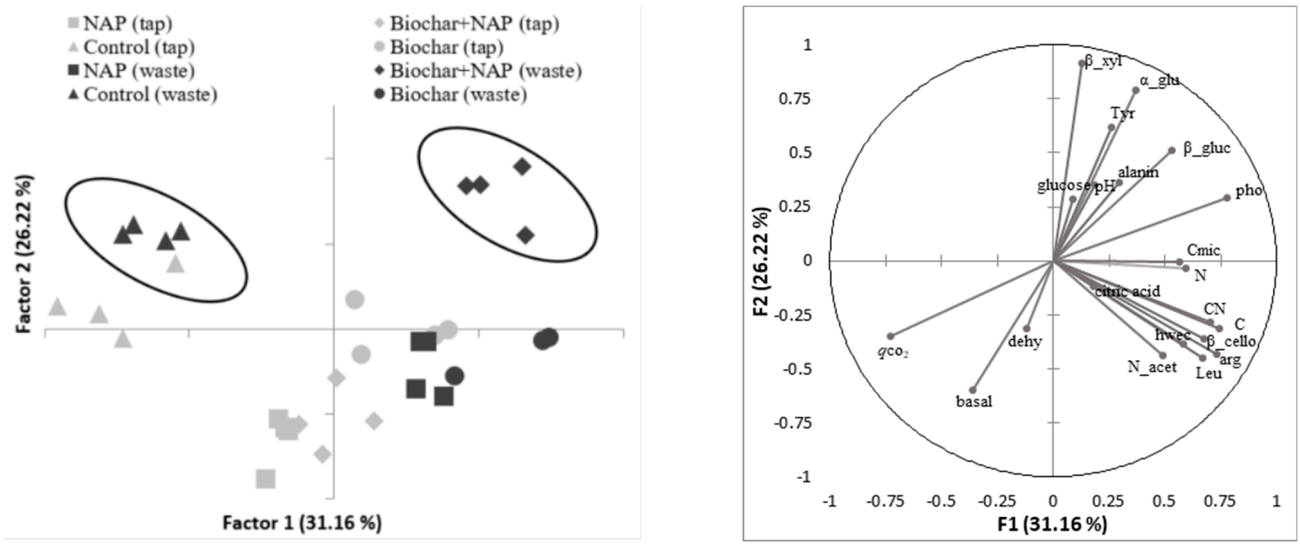
Figure 3. Activity of extracellular enzymatic groups (µmol g^{-1} product h^{-1}) of C-cycle (α-glucosidase, β-glucosidase, β-xylosidase and β-cellobiosidase), N-cycle (leucine-aminopeptidase, tyrosine-aminopeptidase, and arginine-aminopeptidase) and P-cycle (acid phosphatase) in the different soil treatments under tap and wastewater irrigation.

When further looking at individual enzyme activities, we see that biochar alone strongly stimulates β-cellobiosidase activity under both irrigation water qualities, while chitinase and leucine-aminopeptidase are only strongly increased by biochar under tap water (Table 3). Interestingly, water quality effects are absent for acid phosphatase activity in the controls and biochar treatments. However, while NAP additions greatly reduce acid phosphatase activity under tap water, we observed an extreme increase of this enzyme activities in the NAP + biochar treatment with wastewater. Biochar addition under both wastewater and tap water irrigated soil did not influence the intracellular enzyme activities of dehydrogenase ranging between the highest value of 18.5 in the biochar and NAP treatment under tap water and the lowest activity of 12.0 µmol g^{-1} h^{-1} in the same treatment under wastewater irrigation (Table 3).

Table 3. Soil enzyme activities in tap or wastewater irrigated soils. Significant different of means and interactions are given as *p*-values of MANOVA ($p < 0.05$). Values after ± sigh indicate standard deviation with, $n = 4$. Transformation applied after the Shapiro Wilk test are indicated for each parameter. Bc, Fert and Watqu in the table represents biochar, fertilizer and water quality respectively.

		α-Glucosidase	β-Xylosidase	β-Glucosidase	β-Cellobiosidase	N-Acetyl-β-Glucosaminidase	acid Phosphatase	Leucine-Aminopeptidase	Tyrosine-Aminopeptidase	Arginine-Aminopeptidase	Dehydrogenase
		[μmol product g^{-1} soil h^{-1}] ± SD									
Tap water irrigation	Control	16.98 ± 10.98	19.00 ± 1.54	235.60 ± 39.5	1.66 ± 0.47	6.74 ± 1.89	15.95 ± 2.18	3.42 ± 1.58	44.50 ± 15.02	38.72 ± 8.84	15.03 ± 2.96
	Biochar	15.11 ± 7.31	13.76 ± 1.41	132.84 ± 28.77	130.18 ± 22.72	60.86 ± 60.01	34.32 ± 9.28	140.52 ± 30.41	84.86 ± 9.07	426.92 ± 510.04	14.58 ± 3.32
	NAP	6.42 ± 2.87	8.04 ± 3.06	2.10 ± 1.98	29.44 ± 6.00	15.99 ± 3.55	1.63 ± 0.06	24.60 ± 5.45	13.42 ± 4.33	496.91 ± 270.09	15..50 ± 2.98
	NAP + Biochar	8.28 ± 2.88	8.13 ± 1.84	3.43 ± 2.67	4.53 ± 2.76	14.53 ± 6.98	5.68 ± 6.91	38.69 ± 7.54	47.06 ± 9.75	706.57 ± 96.40	18.21 ± 2.86
Waste water irrigation	Control	33.85 ± 6.41	25.58 ± 2.73	254.07 ± 54.42	9.31 ± 7.67	10.23 ± 4.16	17.39 ± 4.89	6.29 ± 0.99	179.16 ± 86.57	322.66 ± 351.08	16.50 ± 1.93
	Biochar	14.96 ± 2.36	12.11 ± 3.25	101.55 ± 9.69	144.82 ± 37.61	134.02 ± 38.36	39.05 ± 2.26	227.28 ± 23.50	80.86 ± 11.48	1657.55 ± 857.	18.50 ± 2.76
	NAP	11.68 ± 5.28	10.11 ± 2.20	95.78 ± 5.41	65.88 ± 51.77	119.59 ± 14.03	38.73 ± 2.20	208.80 ± 17.67	68.78 ± 3.60	1216.10 ± 194.27	14.18 ± 2.28
	NAP + Biochar	36.97 ± 17.77	19.95 ± 2.52	1180.33 ± 26.78	33.75 ± 9.44	8.91 ± 2.26	114.97 ± 3.37	92.50 ± 4.29	108.78 ± 9.28	1111.36 ± 28.80	12.04 ± 2.17
Transformation *p* values from MANOVA		log		sqrt	log	log		sqrt		sqrt	
Block			<0.05								
Biochar (BC)			<0.05	<0.05	<0.05		<0.05	<0.05		<0.05	
Fertilizer (Fert)		<0.05	<0.05	<0.05			<0.05				
Water quality (Watqu)		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		<0.05	
Sig. Interactions ($p \leq 0.05$)		Bc:Fert BC:Fert:Watqu	BC:Fert Fert:Watqu BC:Fert:Watqu	BC:Fert BC:Watqu Fert:Watqu BC:Fert:Watqu	BC:Fert BC:Fert:Watqu	BC:Fert BC:Fert:Watqu	BC:Fert BC:Watqu Fert:Watqu BC:Fert:Watqu	BC:Fert BC:Watqu Fert:Watqu BC:Fert:Watqu		BC:Fert BC:Watqu Fert:Watqu BC:Fert:Watqu	Fert: Watqu

The PCA was used to elucidate the relationship of microbial biomass parameters and measured soil properties. The variabilities of the measured parameters were reduced to two main factors employing a principal component analysis (Figure 4). Both factors explained only 57% of the total variance with factor 1 (31.16% explained variance) dominated by the activity of leucine- aminopeptidase and arginine- aminopeptidases correlating positively with total C and N and labile carbon. Factor 2 (26.22% explained variance) was affected by high factor loadings of C cycling enzymes and showing a negative relation to basal respiration. The respiratory indices were not strongly correlating to any enzyme activity parameter. In the score plot (Figure 4) we found a fairly strong differentiation between wastewater and tap water irrigated samples along both factors. Wastewater irrigated samples are more located in the upper right corner of the plot showing more nutrients and available carbon. The control treatments lie close together and are differentiated on factor 1 from the other treatments.



(**A**) (**B**)

Figure 4. Score plot (**A**) and factor loading (**B**) after varimax rotation with explained variance of components in parentheses (F1: 31.16 and F2: 26.22). Calculated Kaiser–Meyer–Olkin (KMO) index was 0.591. β-xylosidase (β_xyl), α-glucosidase (α_glu), Tyr (tyrosine-aminopeptidase), β_glu (β-glucosidase), pho (acid phosphatase), dehy (dehydrogenase), N acet (N-Acetyl-β-glucosaminidase), Leu (leucine-aminopeptidase), arg (arginine-aminopeptidase), N (nitrogen), C (carbon), CN (C: N), HWEC (hot water extractible carbon), Cmic (microbial biomass carbon), qCO_2 (metabolic quotient).

4. Discussion

4.1. Soil Chemical Parameters

Increased SOC contents have been reported in several studies following wastewater irrigation on a long-term basis [8,27,28]. The non-significant SOC increase in our study (Table 2) was expectable considering the short duration (only one year) of wastewater application which was accompanied by intermittent rainfall inputs. This result confirms the report of Kayikcioglu [29] in a study involving short-term wastewater application where no influences were detected after an irrigation period of three months. The increased SOC content in biochar amended soil as equally mentioned in Häring et al. [4] with values between 1.0 and 2.0 kg m^{-2} SOC is attributed to the high C content of the applied rice husk biochar (42.4%) and correlated with the elevated C/N ratio of soils treated with biochar. Similarly, high labile HWEC in biochar treatments appeared to be due to soluble biochar compounds or to the sorption of soluble SOC compounds that would have otherwise been mineralized or leached. Zimmerman et al. [16] found a suppression of carbon mineralization due to sorption of organic matter by biochar. A similar finding in Heitkötter and Marschner [30] was linked to increased negative charges on biochar surface

through the sorption of aromatic compounds therefore improving microbial resistance of the biochar. In addition, Lu et al. [31] found a negative priming effect with up to 68% reduced decomposition of native soil organic matter due to biochar addition. In their study, the stimulatory effect of N amendments on CO_2 emissions from soil was reduced by biochar addition. The fact that HWEC is mainly from microbial origin [20,32] may explain the relative increase of HWEC in fertilized soil when compared to the unamended control (Table 2). The alleviation of nutrient (N and P) limitations in the relatively poor arable soil may have enhanced microbial turnover of native C [33]. Release of protons during nitrification of fertilizer and negative mass balance of base cation like Ca, K and Mg due to nitrate leaching as explained in Werner et al. [5] on the same field could account for the reduced pH in fertilizer amended soils (Table 2). The total P and N content was influenced by the inorganic NPK fertilizer addition to the NAP plots. The non-significant increase in soil N content after a year of biochar application may be attributed to the low biochar-N (0.6%) content of the applied 20 t ha^{-1} rice husk biochar in our study. Nielsen et al. [34] reported a similar effect of biochar-N on soil N content and therefore mentioned the importance of N enrichment of biochar prior to its application to ensure a significant soil N improvement.

4.2. Basal and Substrate Induced Microbial Respiration

The increased basal respiration in fertilized soil under clean water irrigation suggests an onset stimulation of microbial responds to the supplied mineral N and P nutrients to the inherently poor savannah soil [4]. Gnankambary et al. [35] in a similar study reported fast microbial respiration following an initial application of mineral P and N fertilizer to a relatively poor soil. The reduced basal respiration in wastewater irrigated soils supports the findings of Kayikcioglu [29] who reported a decreased microbial activity in short-term domestic wastewater irrigated soil owing to heavy metals and less availability of C in the wastewater. Other authors like Siebe et al. [27] and Meli et al. [36] contrarily found an increase in basal respiration from soil irrigated with lagoon wastewater for a long period over 15 years. In our short-term study, microbial responds to supplied nutrients from the wastewater might have been delayed as a result of slow activation of dormant soil microbes [7] especially in an onset of a converted rainfed field to irrigated vegetable land use. Incorporation of biochar did not affect basal respiration in our study (Figure 1). However, if basal respiration was normalized to SOC content, biochar decreased SOC-specific basal respiration significantly (data not shown). This suggests, that biochar cannot be utilized as an energy substrate in the same way as the native SOC. This is in line with studies showing that biochar is very recalcitrant in soil and cannot be easily decomposed by microorganisms [37]. Degradation of citric acid in soil is rapid and can contribute to about 20 to 70% of respirable carbon [38], this confirms the elevated respiration in citric acid-induced soils compared to alanine and glucose. Lower alanine induced respiration in fertilized soil compared to unfertilized soil (Figure 1) may suggest profound N mining activity by microorganisms in the unfertilized soil [39]. The addition of biochar had no significant influence on the decomposition of the added substrates. This supports findings from Jones et al. [40] where biochar had no effect on the decomposition of glucose, organic acids or amino acids after either 2 or 3 years of application.

4.3. Microbial Biomass Carbon and Metabolic Quotients

Soil microbial biomass carbon (MBC) was found to be significantly increased by biochar application while wastewater or fertilizer application had no effect (Figure 2A). Other studies, also found an increase in MBC when biochar was mixed into the soil [41–43] and related the findings to the modification provided by biochar such as nutrient and water availability and shielding of microorganisms from predators. However, Dempster et al. [44] found a decrease in MBC with eucalyptus char. There are some concerns about the reliability of soil biological standard methods, i.e., [13] discussed the suitability of common methods to determine soil microbial biomass. Due to the sorption of carbon

compounds to the biochar surface, the chloroform fumigation method could be biased. Liang et al. [41] corrected the measurements with a sorption isotherm for DOC on biochar and reported 21–41% higher values for MBC compared to the standard method. Therefore, the true increase of MBC in our study might even be higher.

Lowered metabolic quotient qCO_2, thus a measure of basal respiration per unit of MBC, in wastewater irrigated soil indicates a more efficient use of organic substrates for microbial catabolism and anabolism [45]. In addition, Heinze et al. [10] found a lower qCO_2 when soils were irrigated with treated wastewater. In biochar amended soils, decreased qCO_2 was observed suggesting higher microbial efficiency similar to other studies of Liang et al. [41] and Zheng et al. [46]. That may be explained by the porous nature of the biochar material providing a habitat for microorganisms or stimulation of microbial growth emanating from the supply of C [47]. Fertilization in NAP treatment elevated qCO_2 in both water qualities indicating some form of stress. This stress was however not observed when biochar was combined with NAP treatment although the pH of the soil samples was similar. We speculate that this might be due to less microbial growth caused by C-limitation or enzyme inhibition [48] in fertilized soils without biochar.

4.4. Enzyme Activities

The activities of microbial extracellular enzymes are an important function that drives nutrient and carbon cycling in the soil ecosystem. In our study, we found a general increase of extracellular enzyme activities in wastewater irrigated soil although reduced basal respiration was measured as indicated by the PCA. This inverse relationship may be explained by the adoption of a substrate-efficient pathway, where low microbial investment is made in nutrient acquisition through enzyme production, this is also indicated by an increased C use efficiency of microbial biomass (qCO_2). Notably, enzyme groups responsible for N hydrolysis (Figure 3) were more active probably due to the input of proteins and other labile N-rich carbon substrates with wastewater irrigation. The result confirms the study of Filip et al. [49] and Chen et al. [11]. They also found higher activities of various enzymes due to long term wastewater irrigation, while Heinze et al. [10] found lower enzyme activities in the top 2 cm of soil irrigated with treated wastewater compared to freshwater control. Amongst the measured extracellular enzymes, β-glucosidase was the most active probably due to its high resistant ability to anthropogenic factors [50] and the major role it plays in the hydrolysis of cellulolytic compounds in the soil [51].

By adding biochar to the unfertilized soil treatments, a diverse reduction in the activity of C-cycling enzymes (β-glucosidase, β-xylosidase, and α-glucosidase) was observed similar to a review by Zhang et al. [52] who reported a reduction of C cycling enzymes in biochar amended soil. The result was linked to an inhibition of C cycling enzymes by high C/N ratio of the biochar [53,54] or the possible suppression of major producer of C cycling enzymes like fungi due to pH enhancement by biochar [55]. Another possible explanation is the sorption of the enzymes to the biochar surface [15] or the blocking of enzyme reaction sites by biochar [13,46].

In contrast to C cycling enzymes, the general increase of N-cycling enzymes (leucine-aminopeptidase, tyrosine-aminopeptidase, and arginine-aminopeptidase) in biochar amended soil is consistent with the study of Wild et al. [56]. High C inputs by biochar might have triggered microbial N mining via the production of N cycling enzymes by responsible microorganisms. For instance, Anderson et al. [57] found a change in abundance of different N cycling bacterial groups and also Zheng et al. [46] showed a higher diversity of the bacterial community in rice paddy biochar addition. Fertilization, however, caused a reduction in the enzyme's activity possibly due to an end-product inhibition whereby the availability of mineral N makes enzymatic N-acquisition less possible [58]. This result can also be linked to the low pH values of the fertilized soil which according to the findings of Paz-Ferreiro et al. [14] can influence enzymatic activity.

Dehydrogenase is an intracellular enzyme that serves as an index for soil microbial activity due to its role in biological oxidation of soil organic matter [59,60]. In support

of Wu et al. [61], we found no biochar effect on dehydrogenase activities. A significant decrease was however reported by Brtnicky et al. [62] whereas other studies [63,64] mention increased dehydrogenase activities in soils with biochar. Although dehydrogenase activities may be explained by several soil conditions, the concern on sorption of enzymes on biochar surfaces may reduce extraction efficiency for its determination [65,66]. However, there is no consensus on how to adjust methods for measuring the enzyme activities in biochar amended soil. The loading pattern of the two main PCA factors in our study showed a distinct influence of nutrient availability and labile carbon on enzymatic performance. The score plots showed distinguished wastewater irrigated plots in a unique position reflecting high nutrient availability most likely in the combined fertilizer and biochar plots.

5. Conclusions

Biochar (20 t ha^{-1}) amendment with wastewater irrigation reduced microbial stress and facilitated more efficient use of carbon in the highly weathered savanna soil. In contrast, the use of mineral NPK fertilizer increased microbial activity but not abundance and elevated carbon losses from soil. Biochar protected carbon in soil from decomposing and therefore sequester SOC as evident by decreased basal respiration and increased HWEC. Total enzymes activity was more profound under wastewater irrigation compared to tap water. In addition, biochar treated soil showed a higher diversity of enzymes involved in C and N cycling suggesting a more diverse microbial function. Activities of C-cycling enzymes were reduced whereas an increase in N-cycling enzymes were observed with biochar addition. Wastewater irrigation and biochar were both found to be beneficial for soil microorganisms, therefore having positive effects on soil fertility.

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