Fritz *et al. Chem. Biol. Technol. Agric.* (2022) 9:48
https://doi.org/10.1186/s40538-022-00312-4

Chemical and Biological Technologies in Agriculture

RESEARCH Open Access

The combined application of nitrogen and biochar reduced microbial carbon limitation in irrigated soils of West African urban horticulture

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Abstract

Background: Intensive wastewater irrigated urban horticulture in sub-Saharan West Africa receives high nutrient inputs, which lead to large gaseous and leaching losses. The addition of biochar to the usually sandy soils may reduce these losses and improve the habitat conditions for soil microorganisms. Two similar experiments focused on crop yields and nutrient balances have been carried out over a 2-year period in semi-arid Ouagadougou, Burkina Faso, and in sub-humid Tamale, Ghana, representing to some extent different but typical locations in West Africa.

Methods: Biochar and N fertilization effects were measured on soil microbial biomass carbon (MBC), fungal ergosterol, and functional diversity, estimated by multi-substrate-induced respiration. It was additionally possible to study the effects of clean water irrigation on the respective microbial properties in Tamale soil.

Results: Sole biochar addition did not affect any soil chemical or soil biological properties analyzed. In contrast, biochar application with N fertilization increased the mean respiratory response of the 11 substrates added by 23% in the Ouagadougou soil and by 13% in the Tamale soil. N fertilization decreased soil pH in both cities by 1.1 units. However, a pH-H_2O of 4.7 led to reduced MBC and ergosterol contents at Tamale. Also, the Shannon index of the respiratory response was positively correlated with the soil pH. Clean water irrigation decreased the ergosterol content and increased the respiratory response to organic acids.

Conclusions: Biochar addition with N fertilization improved habitat conditions for soil microorganisms. An N fertilizer-induced decline in soil pH < 5 should be avoided, as it decreased MBC and microbial functional diversity.

Keywords: Vegetable production, Wastewater irrigation, Microbial biomass, Functional diversity, Acidification

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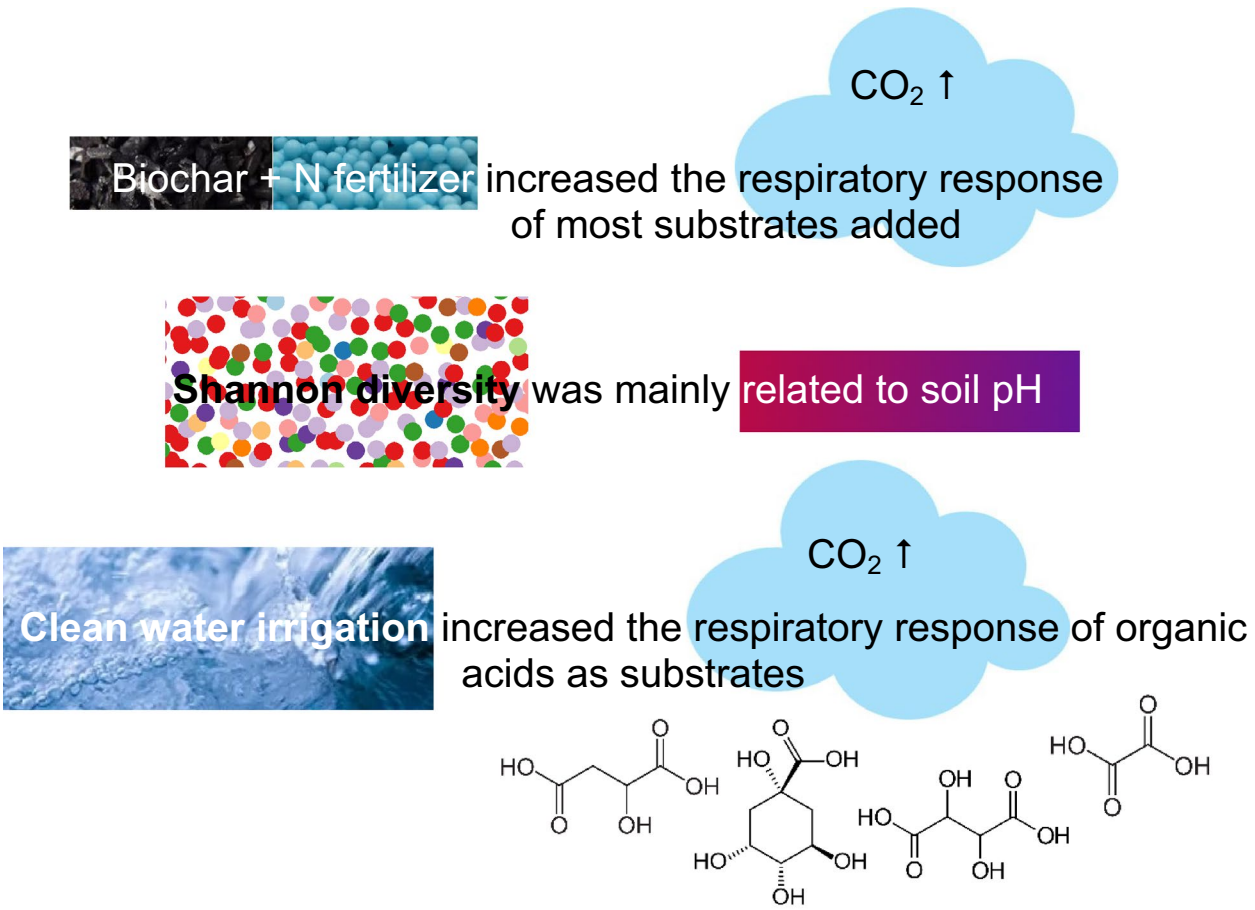
Graphical Abstract



Background

Sub-Saharan West African countries, such as Burkina Faso and Ghana, are exposed to increasing urbanization, accompanied by the expansion of urban agriculture [1, 2]. In contrast to rural agriculture, urban horticulture in sub-Saharan West African countries receives considerable organic inputs by mulching of harvest residues, fertilization with organic manures, and irrigation with wastewater [3–5]. Such intensively used systems lead to large gaseous and leaching losses of nutrients [4, 6]. The addition of biochar to soil may reduce these losses and the cultivation of square meters instead of hectares makes it possible to add biochar in a meaningful quantity to improve soil fertility [7].

Biochar is produced by pyrolysis of harvest residues with restricted O_2 supply [8]. Biochar properties strongly depend on the substrates and pyrolysis conditions [9]. Carbonized biochar usually consists of a polycyclic aromatic structure, which is highly stable in soil [9, 10] and, thus, more resistant against microbial decomposition than soil organic C (SOC) [11]. However, biochar effects on soils properties strongly depend on soil type, especially clay content and soil pH [12], but may also be affected by climate [13]. Biochar has been reported to increase the water holding capacity and, thus, water storage for crop use in highly acidic Ferralsols of humid tropical regions [14, 15]. In this context, Steiner et al. [16] observed a linear relationship between increased microbial biomass carbon (MBC) contents and biochar application rates in Amazonia.

Biochar effects might be different in sandy soil under the semi-arid to sub-humid climate of sub-Saharan West Africa. This is characterized by an 8-month dry period that requires irrigation for year-round production in urban horticultural systems, which is usually supplied as domestic wastewater [17]. This wastewater use for irrigation has the advantages of reducing the demand for sewage plants [18], of supplying nutrients to vegetables

Table 1 Mean yield, N-input, P-input at the experimental sites in Ouagadougou (Burkina Faso) and Tamale (Northern Ghana) over the experimental years 2014 to 2016 taken from Akoto-Danso et al. [4]

Treatment	Yield	N-input (kg ha^{-1} ± SD)	P-input
Ouagadougou			
Control-WW	3370 ± 757	31 ± 18	6 ± 3
FP-WW	9246 ± 3356	675 ± 279	139 ± 13
FP + BC-WW	9553 ± 3183	672 ± 285	146 ± 22
Tamale			
Control-WW	4116 ± 737	239 ± 43	76 ± 43
Control-CW	1345 ± 733	4 ± 1	0 ± 1
FP-WW	6308 ± 2209	440 ± 123	135 ± 27
FP-CW	4143 ± 2011	205 ± 129	59 ± 35
FP + BC-WW	6527 ± 2546	440 ± 123	139 ± 30
FP + BC-CW	4749 ± 2526	205 ± 129	63 ± 44

In Ouagadougou, 5 crops were harvested in the rainy and 6 in the dry seasons. In Tamale, 7 crops were harvested in the rainy and 6 in the dry seasons. Respective data for the BC treatment were not provided in any previously published study from the experimental sites

SD standard deviation, *CW* clean water irrigation, *WW* wastewater irrigation, *BC* biochar, *FP* fertilization according to farmers' practice

[17, 19], and of saving scarce freshwater resources [20]. However, wastewater contains not only fecal pathogens [21–23], but also heavy metals, [24], organic pollutants [25], and NaCl [18], which cause problems for food safety, human health, and soil fertility [26]. Biochar application to soil may reduce the negative effects of wastewater application on the environment by absorbing these pollutants [9, 12], which most likely increases the functional diversity of the soil microbial community.

The effectts of biochar and N fertilization were investigated in two wastewater irrigated field experiments at semi-arid Ouagadougou (Burkina Faso) and sub-humid Tamale (Northern Ghana) [4, 5]. These experiments were focused on yields and nutrient balances in vegetable production systems over a 2-year period comprising 11 and 13 crops, respectively. Effects of N fertilization according to farmers' practices, addition of biochar made from rice husks and corn cobs as well as wastewater and clean water irrigation on dry and fresh matter yields were extensively studied [4, 5]. Also, the effects of these treatments on changes in chemical soil properties such as soil pH, cation exchange capacity, SOC, and total N as well as extractable phosphorus have been carefully monitored at Ouagadougou and Tamale [3]. However, soil microorganisms as drivers of plant residue decomposition as well as C and N mineralization have been completely neglected in these experiments.

The biomass of soil microorganisms is an important indicator for soil fertility in tropical and sub-tropical soils [27], because MBC draws a relationship between plant C input, SOC stocks, and microbial mobilization–immobilization turnover of nutrients [28, 29]. MBC is usually combined with basal respiration rate, i.e., SOC mineralization in the absence of fresh substrates [30–32]. The membrane component ergosterol [33] gives additional important information on the contribution of saprotrophic fungi to the microbial community of agricultural soils [34, 35]. However, MBC often failed to indicate the rather subtle biochar effects [36, 37]. Multi-substrate-induced respiration (MSIR) is an interesting additional approach [38, 39]. MSIR is a potential activity of whole microbial communities in the period immediately after adding low molecular weight organic substances to soil before microorganisms start to grow [40]. Consequently, more of these substances will generally be anabolized by a C-limited microbial community, whereas an N-limited community is specifically characterized by the anabolization of N-containing substrates, such as amino acids or amino sugars. Consequently, MSIR may be able to create a link between functional diversity and a mechanistic understanding of soil processes.

The objective of the current study was to fill the current knowledge gap in two extensively studied experiments by measuring MBC, MSIR, and ergosterol. The study was designed to investigate the following hypotheses: (1) the sole application of biochar has no effects on soil microorganisms, because the effects on crop yield are negligible [4]. (2) The combined application of biochar and N fertilization increases MBC, fungal biomass, and functional diversity, because the negative effects of the nitrification-induced pH decline are reduced [3]. (3) Relative to clean water, wastewater irrigation may not have any impact on microbial biomass and functional diversity, as positive and negative impacts counterbalance each other. Effects of water quality on soil microorganisms were only studied in the Tamale soil, due to the strong differences in nutrient concentration between clean and wastewater in this city [3, 4], leading to marked differences in crop yield.

Material und methods

Study sites

The study was carried out in two West African cities, Ouagadougou (12°24′16 N, 1°28′40 W; approx. 1,500,000 inhabitants), the capital city of Burkina Faso, and Tamale (9°28′29 N, 0°50′53 W; approx. 300,000 inhabitants), the capital city of the Northern Region of Ghana, which are both characterized by a climate with a unimodal rainy season. The long-term average annual rainfall is 788 mm at Ouagadougou and 1111 mm at Tamale. In both cities, rainfall is lowest in January and highest in August and

September. During the study period (2014–2016), the mean annual temperature was 28.2 °C in Ouagadougou and 27.5 °C in Tamale, measured by a watchdog weather station (Spectrum Technologies, Aurora, USA). The soil at semi-arid Ouagadougu was characterized as Cutanic Haplic Lixisol [41] with 60% sand, 35% silt and 5% clay. The soil at sub-humid Tamale was a Petroplinthic Cambisol with 46% sand, 48% silt and 6% clay.

Until the start of the experiment, the Ouagadougou site had been used for intensive vegetable horticulture, while the Tamale site had been used for rainfed maize (*Zea mays* L.) production. The cropping pattern in the two cities was similar during the experimental period for 11 and 13 harvest events (Table 1). The Ouagadougou site was cultivated with lettuce (*Lactuca sativa* L.), cabbage (*Brassica oleracea* L.), amaranth (*Amaranthus cruentus* L.), jute mallow (*Corchorus olitorius* L.), roselle (Hibiscus sabdariffa L.), and carrots (*Daucus carota* subsp. *sativus* (Hoffm.) Schübl. & G. Martens). The Tamale site was cultivated similarly, except for a start with maize (*Zea mays* L.) and a repeat of jute mallow after its first cultivation. All plots were irrigated with watering cans once or twice a day with a predefined quantity of water, which reflected farmers' perception of the weather-related water demand of the current crops. Precipitation was considered for irrigation in the rainy seasons.

Experimental design, setup, and treatments

The two experiments were carried out with four treatments as described in detail [3–5, 17] on plots of 2 × 4 m, which were replicated four times in a split-block design: (1) unfertilized control, (2) only biochar (BC), (3) fertilizer application according to local farmers' practice (FP), and (4) FP + biochar. Generally, in Ouagadougou a combination of urea (46% N: 70–375 kg ha^{-1} per crop) and cattle manure (9 to 20 t ha^{-1} per crop with 17% C and 1.3% N) was used (Table 1). The fertilization in Tamale comprised only NPK (15-15-15: 200–563 kg ha^{-1} per crop). All treatments were also irrigated according to local farmers' practice with wastewater (WW), obtained from a large open channel at Ouagadougou and from an untreated sewage channel at Tamale. Wastewater contained more N and P in Tamale: 31.9 mg N, 8.9 mg P, and 8.4 mg K l^{-1} than in Ouagadougou: 3.9 mg N, 0.8 mg P, and 44 mg K l^{-1} [3]. In Tamale, untreated wastewater

Table 2 Mean soil pH and mean contents of SOC, total N, MBC, and ergosterol, mean basal respiration rate and metabolic quotients qCO_2 in soil from the experimental sites at Ouagadougou (Burkina Faso) and Tamale (Northern Ghana); probability values for the two-way ANOVA at Ouagadougou and the three-way ANOVA at Tamale, interactions were all not significant with one exception at Tamale

Treatment	Soil pH (H_2O)	SOC (mg g^{-1} soil)	Total N	MBC (µg g^{-1} soil)	Ergosterol	CO_2-C (µg d^{-1} g^{-1} soil)	qCO_2 (mg CO_2-C d^{-1} g^{-1} MBC)
Ouagadougou							
Control	8.1	5.7	0.58	113	0.06	8.5	74
BC	8.0	8.0	0.53	114	0.04	8.8	81
FP	6.9	8.1	0.99	136	0.13	9.8	71
FP + BC	7.0	12.8	1.10	168	0.03	11.5	71
Probability values							
Biochar	NS	< 0.01	NS	NS	NS	NS	NS
Fertilizer	< 0.01	< 0.01	< 0.01	NS	NS	NS	NS
CV (± %)	2.6		19	30 170	52	32	20
Tamale							
Control	5.9	4.2	0.40		0.23	10.0	63
BC	5.8	7.1	0.46	173	0.21	9.9	57
FP	4.7	4.7	0.45	72	0.13	8.8	125
FP + BC	4.7	7.5	0.52	100	0.16	9.8	108
Probability values							
Biochar	NS	< 0.01	0.02	NS	NS	NS	NS
Fertilizer	< 0.01	NS	NS	< 0.01	0.01	NS	< 0.01
Irrigation	NS	NS	NS	NS	0.01	NS	NS
F × I	NS	NS	NS	NS	NS	0.01	NS
CV (± %)	2.2	15	15	30	52	16	24

CV mean coefficient of variation between replicate samples ($n = 4$), *NS* not significant, *FP* fertilization according to farmers' practice

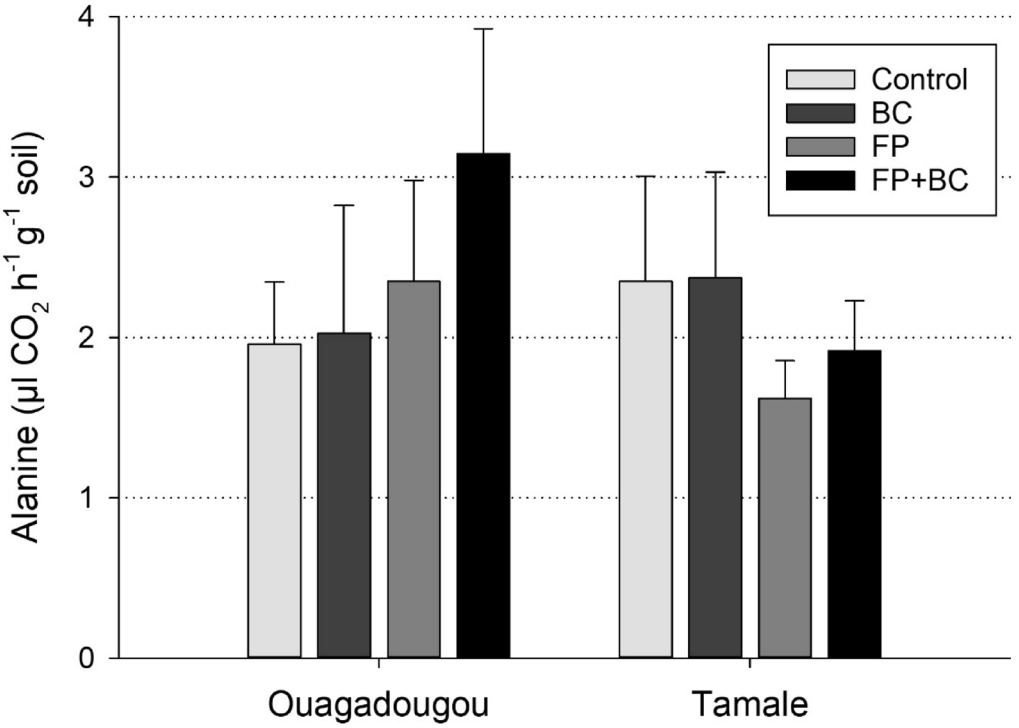


Fig. 1 Mean alanine-induced respiration rates in soil from the experimental sites in Ouagadougou (Burkina Faso) and Tamale (Northern Ghana); probability values for the two-way ANOVA at Ouagadougou: Biochar (not significant) and Fertilizer ($P < 0.04$); probability values for the three-way ANOVA at Tamale: Biochar (not significant), Fertilizer ($P < 0.01$), and Irrigation (not significant); interactions were all not significant; bars on top of each column indicate one standard deviation

(WW) was compared with clean water (CW), obtained from a tap: 0.4 mg N, 0.1 mg P, and 1.7 mg K l^{-1} [3].

Biochar was produced by slow pyrolysis using a local kiln at a temperature of about 500 °C [7]. Biochar was made from rice husks in Tamale and from corn cobs at Ouagadougou. Biochar from corn cobs had a pH-$CaCl_2$ of 10.3, with concentrations of 68% C, 0.9% total N, and 19% ash. Biochar from rice husks had a pH-$CaCl_2$ of 9.1, with concentrations of 42% C, 0.6% total N, and 45% ash [3]. A single biochar application in May 2014 at a rate of 20 t ha^{-1} was used in a 2-year study [17] and incorporated at 0–20 cm depth in both cities. After incorporation, the soil of all plots was thoroughly tilled.

Soil analysis

One soil sample was taken with an auger (7 cm diameter) after harvest of lettuce in June 2016 at 0–20 cm, sieved (< 2 mm), and stored at 4 °C in polyethylene bags until analysis. Soil pH was measured in water at a 1:2.5 soil to H_2O ratio. Soil was dried at 80 °C and ground with a ball mill before measuring total C and N, using a Vario MAX CN analyser (Elementar, Hanau, Germany), ergosterol was extracted from 2 g moist soil with 100 ml ethanol for 30 min by oscillating shaking at 250 rev min^{-1} [42]. Then, ergosterol was measured by reversed phase HPLC (Gynkotek M 480 pump, UVD 340 S detector, and Gina 50 autosampler, Germering, Germany), using 100% methanol as the mobile phase and detected at a wavelength of 282 nm.

Microbial functional diversity

Functional diversity of soil microorganisms was determined by the MSIR approach using the MicroResp™ method [38]. The soil was adjusted to a water holding capacity of 45%, before weighing 300 mg into each deep well (1.1 ml) of a microtiter plate (Nunc, Thermo Electron LED, Langenselbold, Germany) and stored for 3 days in the dark at 25 °C prior to MSIR analysis. The physiological profiles were determined by applying distilled water (for basal respiration), three amino acids [L-alanine (Ala), L-glutamine (GluN), and L-serine (Ser)], one amino sugar [N-acetyl-glucosamine (NAG)], two carbohydrates [(D-glucose (Glc), D-fructose (Fruc)], four carboxylic acids [malic acid (Mal), quinic acid (Qui), oxalic acid (Oxa), and tartaric acid (Tat)], and one phenolic organic acid [protocatechuic acid (ProC)]. These substrates were chosen to present a cross section of root

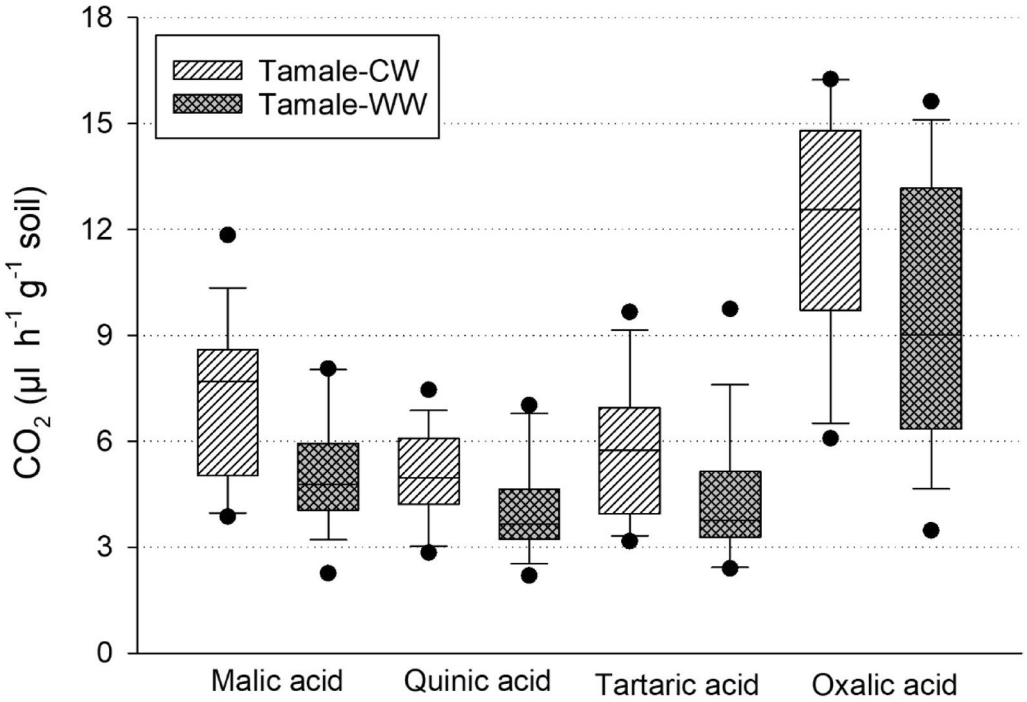


Fig. 2 Boxplots of respiration rates, induced by malic acid, quinic acid, tartaric acid and oxalic acid in soil from the experimental site in Tamale (Northern Ghana)

exudates [38] and microbial components and products [43, 44]. A substrate concentration of 8 mg g^{-1} dry soil was used by placing 20 µl of solution in the deep well plate, before incubating the soil for 6 h at 25 °C. Only 1 mg g^{-1} soil of L-glutamine and 0.3 mg g^{-1} soil of protocatechuic acid were used, due to their low solubility at higher concentrations. An excess of substrate was always added to the soil to saturate the microbial community, as tested in previous studies [38, 39].

A colorimetric CO_2 trap [38] was stored for 72 h in a closed PVC bag, containing soda lime and wet tissue paper. The color of the CO_2 trap was measured immediately before sealing and after 6 h of incubation (25 °C) at 572 nm (FLUOstar, BMG, Offenburg, Germany). The CO_2 trap was calculated as µl $CO_2 = 51 \times (0.2 + \mathrm{ABS})^3$ [40], where ABS is the difference in absorption of T1 and T0. The Shannon diversity index was calculated using the formula $H = -\Sigma$ pi (ln pi), where pi is the particular activity of the sum of all activities [45]. In addition, basal respiration was calculated from aqua dest. addition and MBC from Glc addition ($30 \times$ µl CO_2 g^{-1} h^{-1}) [46].

Statistical analysis

Data are presented as arithmetic means on a dry weight basis. Statistical analyses were carried out using SigmaPlot 13.0 (Systat, San José, USA). Data were tested for normality of residuals using Shapiro–Wilk test. In case of non-normality, data were ln-transformed. The significance of treatment main effects was analyzed by a two-way ANOVA for the samples from Ouagadougou and a three-way ANOVA for the samples from Tamale. Biochar and N-fertilization on the mean substrate-specific respiration were assessed using a paired t-test. Discriminant function analysis was conducted on the combined MSIR data for all samples from Ouagadougou ($n = 16$) and Tamale ($n = 24$) to investigate fertilizer effects on the substrate utilization patterns, with SPSS 16.0 statistical software (SPSS 16.0). To describe discrimination, substrate-specific respiration was correlated to the canonical scores of the significant ($P < 0.05$) discriminant functions (DF). Pearson correlation coefficients were used to express significance. The canonical DF scores were correlated with the soil parameters to identify their contribution to discrimination.

Results

Biochar addition increased SOC contents in both cities and total N contents at Tamale (Table 2), but no other soil chemical or soil biological property analyzed (Table 3). Nitrogen fertilization decreased soil pH in both cities by 1.1 units (Table 2), so that a pH-H_2O of 4.7 was reached at Tamale. This strong acidification was combined with

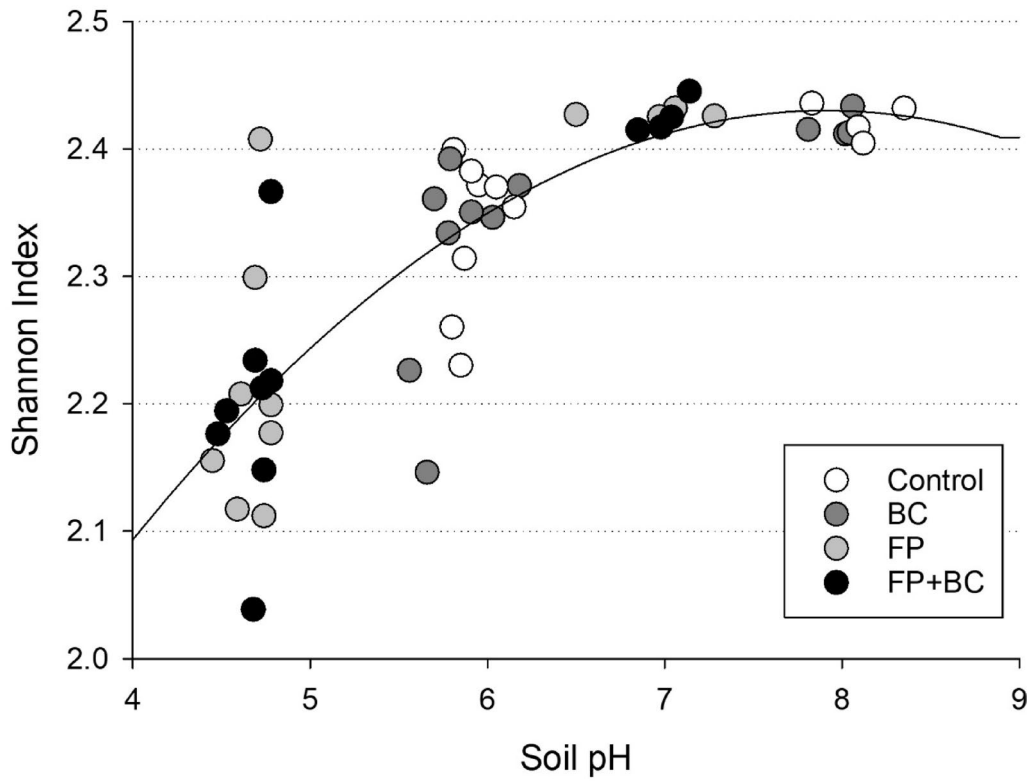


Fig. 3 Relationship between Shannon diversity index and pH in soil from the experimental sites in Ouagadougou (Burkina Faso) and Tamale (Northern Ghana)

a significant reduction in MBC and ergosterol contents, whereas basal respiration was not affected. This led to increased qCO_2 values in comparison with the unfertilized control treatments.

Without N fertilization, Mal, Qui, Oxa, and Tat exhibited on average a 90% ($P<0.01$, paired t-test) stronger respiratory response in the Tamale soil than in the Ouagadougou soil (Table 3), whereas all other substrates led to similar respiration rates in both soils. With N fertilization, Ala (Fig. 1) and Mal-induced respiration was significantly increased by 29% and 19%, respectively, in Ouagadougou soil, whereas Ser, GluN, NAG, Qui, Oxa, Tat, Pro, and Fruc-induced respiration remained largely unaffected (Table 3). In contrast, Ala (Fig. 1), Ser, GluN, NAG, Pro, and Fruc-induced soil respiration was significantly decreased with N fertilization in Tamale soil (Table 3), whereas the four organic acids Mal, Qui, Oxa, and Tat again remained unaffected.

Biochar application increased the mean respiratory response of the 11 substrates added by 23% ($P<0.01$, paired t-test) in Ouagadougou soil and by 13% ($P<0.02$, paired t-test) in Tamale soil only in combination with N fertilization. Clean water irrigation in Tamale decreased basal respiration of soils without N fertilization, whereas the reverse effect was observed with N fertilization, leading to the only significant second-order interaction. Clean water irrigation decreased the ergosterol content at Tamale (Table 2), but increased organic acid-induced respiration (Fig. 2).

The Shannon index of the respiratory response to water and the 11 substrates added was generally positively correlated with soil pH (Fig. 3). This index varied in a small range around 2.42 at Ouagadougou and a much larger range between 2.05 and 2.41 at Tamale. There, biochar addition increased the range of the Shannon index without N fertilization and reduced the range with N fertilization. This different behavior was partly reflected by the DF2 (Fig. 4), where the pure BC treatment produced negative scores and the FP + BC treatment yielded positive scores. However, N fertilization effects were much more clearly separated by DF1, which explained 81% of the variance.

Mal, Qui, Oxa, and Tat were the only substrates that were negatively correlated with soil pH and the Shannon index (Table 4), whereas Ser, GluN, NAG, Pro, and Fruc were positively correlated. The four organic acids Mal, Qui, Oxa, and Tat remained unaffected by SOC content, whereas all other substrates showed highly significant

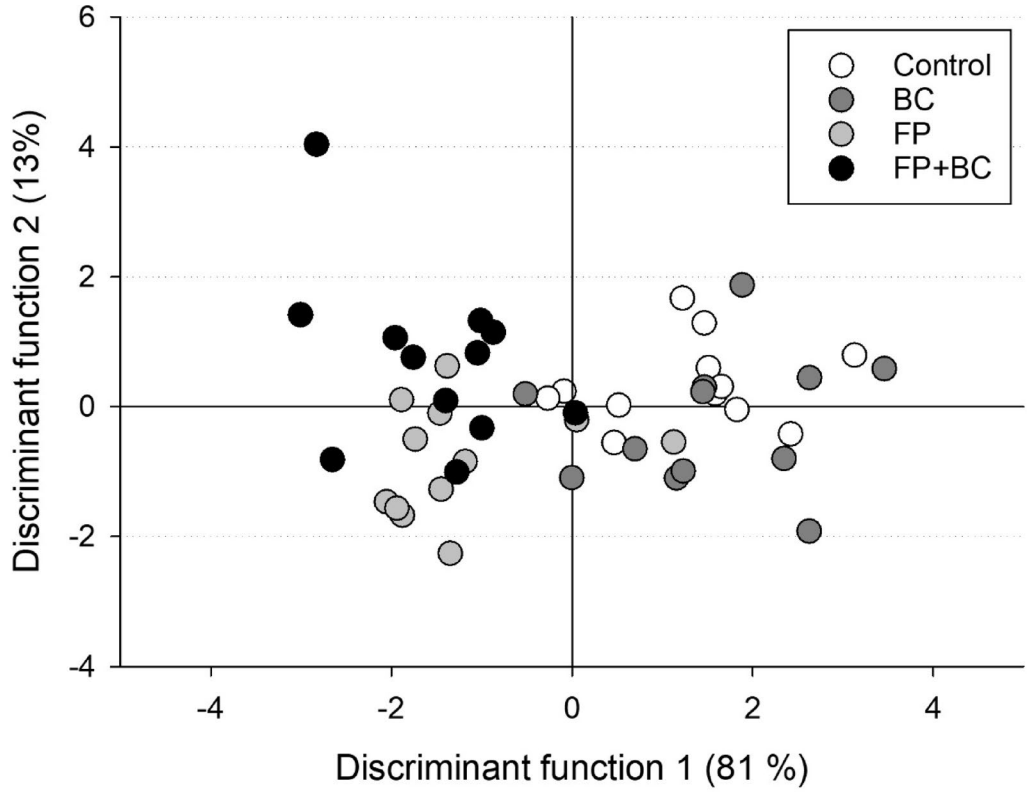


Fig. 4 Discriminant function analysis of catabolic response of 11 substrates plus aqua dest. for the four fertilizer treatments in soil from the experimental sites in Ouagadougou (Burkina Faso) and Tamale (Northern Ghana)

correlations. N effects explaining DF1 were positively correlated with GluN, Pro, Fruc, and Glc (Fig. 4), whereas biochar effects explaining DF2 were significantly correlated with aqua dest., Ala, Ser, NAG, Mal, Pro and Glc.

Discussion

Sole biochar addition to soil did not affect biomass or functional diversity of soil microorganisms in either of the cities. Also, the effects on crop yield were generally negligible and only transient for short times if some increases occurred [4, 5, 17]. Consequently, the sole biochar treatment was not considered in a publication summarizing treatment effects on dry matter yields in Ouagadougou and Tamale (Table 1; [4]).

In contrast, biochar addition in combination with N fertilization increased the mean respiratory response of soil microorganisms to virtually all substrates added to soil in both cities in comparison with the sole N fertilization treatment, despite the absence of significant effects on a single substrate. A higher respiratory response indicates a reduction in microbial C limitation [40], e.g., due to an increased C input into soil by harvest and root residues of the crops cultivated, assuming a relationship between crop yield and belowground biomass [47]. However, the reduction in microbial C limitation was not strong enough to increase MBC contents. The increase in respiratory response was markedly stronger in the alkaline Ouagadougou, due to the strong fertilizer-induced increase in MBC, than in the acidic Tamale soil, where fertilization further reduced soil pH accompanied by a strong decline in MBC. This contradicts the view that biochar especially improves microbial habitat conditions in acidic low fertility soils, such as a Ferralsol in Brasilia [48]. Fertilization with inorganic N and organic manure increased total N by 88% and SOC by 52% in the alkaline Ouagadougou soil in comparison with the control and sole BC treatment. These increases were accompanied by a significantly higher respiratory response to alanine, which indicates a strong reduction in N limitation [40], as otherwise alanine should have been anabolized to microbial metabolites and not catabolized to CO_2.

Inorganic N fertilization with ammonium generally decreased soil pH in both cities, which is most likely due to nitrification [3]. This was of little relevance in the alkaline Ouagadougou soil, but it is a serious threat to soil fertility in the acidic Tamale soil, where inorganic fertilization reduced the pH to below the critical value of pH 5. At this pH, soluble and exchangeable Al^{3+} is a serious

Table 3 Mean multi-substrate-induced respiration rates in soil from the experimental sites in Ouagadougou (Burkina Faso) and Tamale (Northern Ghana); probability values for the two-way ANOVA at Ouagadougou and the three-way ANOVA at Tamale, interactions were all not significant

Treatment	Ser	GluN	NAG	Mal	Qui	Oxa	Tat	Pro	Fruc
	(μl CO_2 h^{-1} g^{-1} soil)								
Ouagadougou									
Control	2.8	3.6	2.3	3.8	3.1	3.5	2.9	2.3	3.6
BC	3.0	3.5	2.4	3.4	3.1	3.9	2.8	2.2	3.2
FP	3.7	4.1	3.0	3.6	3.3	3.5	2.8	2.7	4.1
FP + BC	4.5	5.0	3.7	4.3	3.9	4.5	3.5	3.1	5.1
Probability values									
Biochar	NS	NS	NS	NS	NS	NS	NS	NS	NS
Fertilizer	0.06	NS	NS	0.03	NS	NS	NS	0.08	NS
CV (±%)	30	26	26	27	33	26	32	24	30
Tamale									
Control	3.1	3.4	2.7	6.7	4.7	10.1	5.7	2.7	4.7
BC	3.1	3.8	2.7	6.6	4.6	10.9	5.7	2.7	5.0
FP	1.9	1.7	1.6	4.9	4.3	10.4	4.5	1.5	1.9
FP + BC	2.1	1.7	2.0	6.2	4.5	11.4	4.6	1.8	2.2
Probability values									
Biochar	NS	NS	NS	NS	NS	NS	NS	NS	NS
Fertilizer	< 0.01	< 0.01	< 0.01	NS	NS	NS	NS	< 0.01	< 0.01
Irrigation	NS	NS	NS	0.01	0.06	0.05	0.03	NS	NS
CV (±%)	27	29	22	35	31	36	39	20	24

CV mean coefficient of variation between replicate samples ($n = 4$), *NS* not significant, *FP* fertilization according to farmers' practice

Table 4 Pearson correlation coefficient between substrate utilization and discriminant functions DF1 and DF2, Shannon index, soil pH, and SOC in soil from the experimental sites in Ouagadougou (Burkina Faso) and Tamale (Northern Ghana)

	DF1	DF2	Shannon index	Soil pH	SOC
Aqua	NS	0.34*	NS	NS	0.50**
Ala	NS	0.46*	NS	NS	0.58**
Ser	NS	0.31*	0.60**	0.45**	0.53**
GluN	0.41**	NS	0.66**	0.60**	0.50**
NAG	NS	0.36*	0.43*	0.41*	0.57**
Mal	NS	0.36*	−0.45**	−0.41*	NS
Qui	NS	NS	−0.39*	−0.35*	NS
Oxa	NS	NS	−0.80**	−0.68**	NS
Tat	NS	NS	−0.40*	−0.37*	NS
Pro	0.40**	0.39*	0.49**	0.43*	0.45**
Fruc	0.54**	NS	0.45**	0.38*	0.41**
Glc	0.47**	0.41**	0.32*	NS	0.34*
DF1				0.34*	NS
DF2				NS	0.30*

NS not significant

*$P < 0.05$; **$P < 0.01$

threat to crops [49] and soil microorganisms, leading to a strong decline in MBC, respiratory response, and functional diversity. The negative pH effects on MBC and microbial functional diversity override the positive effects of increased crop yields, probably accompanied by increased input of harvest and root residues. This is in line with the view that soil pH is the dominating factor that controls microorganisms in soil [28, 50, 51]. The significantly increased qCO_2 values indicate the higher demand for maintenance energy in acidic soil [31, 52], strongly impeding SOC accumulation and, thus, soil fertility [28]. Care should be taken to avoid such N fertilizer-induced acidification, not only in West African urban horticulture, but also elsewhere.

Irrigation with wastewater had no general negative effects on MBC in the acidic Tamale soil, but a strong positive effect on ergosterol, an important indicator for saprotrophic fungi in agricultural soils [34, 35]. The positive biochar effect on fungi might be due to their stronger ability to break down the recalcitrant C components in wastewater compared with bacteria [53–55]. A striking feature of the current results is the response of the malic acid, quinic acid, oxalic acid, and tartaric acid to differences in soil properties between Ouagadougou and Tamale as well as to differences between clean water and wastewater. This is even more remarkable as these four organic acids were the only ones that did not respond to N fertilization and that were not correlated

with discriminant functions. The strong response of the four organic acids is probably due to their extremely high respiratory quotient (mol CO_2/mol O_2), which results in higher CO_2 evolution rates during catabolization in comparison with other substrates [56, 57].

In the acidic Tamale soil, the respiratory response to the addition of organic acids is nearly twice that in the alkaline Ouagadougou soil. One reason might be a stronger adsorption of organic acids at high pH [58]. However, the respiratory responses to organic acid addition were like the other substrates in all treatments, i.e., also in the N fertilization treatments with neutral pH. Another reason might be a stronger adaptation of the microbial community to mineralize organic acids in their presence [59]. However, the catabolic use of organic acids probably requires less adaptation than the anabolic use, which seems to be higher in the Ouagadougou soil, as indicated by the lower respiratory responses to organic acid addition. In this alkaline soil, crop roots, especially those of non-mycorrhizal cabbage and lettuce [60], might excrete more organic acids for mobilizing phosphate. Consequently, these acidic organic components are more common for microbial anabolism, as indicated by the lower respiratory response to organic acid addition in the alkaline Ouagadougou soil.

The higher respiratory response of soil microorganisms to organic acid addition in the clean water treatment at Tamale might also be due to the lower presence of organic acids in comparison with wastewater. Another reason might be differences in microbial community structure, as the soil of the clean water treatments contained fewer saprotrophic fungi, which seem to have a higher ability to excrete and to anabolize organic acids than bacteria [61]. This would be an appropriate objective for a follow-up experiment.

Conclusions

Sole biochar addition to field plots in Ouagadougou and Tamale did not affect soil microbial biomass carbon (MBC) or functional diversity, estimated by multi-substrate-induced respiration. Biochar addition with N fertilization generally increased the microbial respiratory response to most substrates added, indicating a reduction in C limitation for soil microorganisms and, thus, improved habitat conditions. However, the N fertilizer-induced decline in soil pH < 5 should be avoided, as it decreased MBC and microbial functional diversity in the Tamale soil. Only the respiratory response to organic acids added remained unaffected by biochar and N fertilizer addition but was significantly increased by clean water compared with wastewater irrigation. This is an interesting feature of the current results, which was accompanied by a reduction in saprotrophic soil fungi. However, the long-term effects of this shift in microbial biomass and functional diversity in soil needs further evaluation to promote best management practices of biochar application and wastewater use.

Abbreviations

SOC: Soil organic carbon; MBC: Microbial biomass carbon; MSIR: Multi-substrate-induced respiration; Ala: L-Alanine; GluN: L-Glutamine; Ser: L-Serine (Ser); NAG: One N-acetyl-glucosamine; Glc: D-Glucose; Fruc: D-frUctose; Mal: Malic acid; Qui: Quinic acid; Oxa: Oxalic acid; Tat: Tartaric acid; ProC: Protocatechuic acid.

Acknowledgements

The technical assistance of Gabriele Dormann, Martin Lüttge, and Larissa Krause in soil analysis is highly appreciated.

Author contributions

ALF: analyzed the soil samples, conducted literature review, data analysis and draft manuscript preparation. RJ: contributed to laboratory experiments, data analysis, and reviewed the draft manuscript. RB: contributed to laboratory experiments and reviewed the draft manuscript. CS: organized the field experiment, took the soil samples, and reviewed the draft manuscript. AB: conceived funding, designed the study, and reviewed the draft manuscript. RGJ: supervised analysis, contributed to data analysis and wrote the final manuscript. All authors read and approved the final manuscript.

Funding

Open Access funding enabled and organized by Projekt DEAL. The field work of this study was funded by the German Ministry of Education and Research (BMBF) within the framework of the UrbanFood^{Plus} project (www.urbanfoodplus.org) as part of the GlobE initiative (BMBF, FKZ 031A242A).

Availability of data and materials

The data are available on request.

Declarations

Competing interests

The authors declare that they have no competing interests.

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Received: 6 December 2021 Accepted: 21 June 2022
Published online: 16 July 2022

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