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Effect of ramial chipped wood and poultry manure amendments on soil chemical properties and fungal communities in Benin

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ABSTRACT

This study investigated effects of soil amendments with ramial chipped wood (RCW) derived from *Gmelina arborea* and *Sarcocephalus latifolius*, together with poultry manure, on soil chemical properties and fungal communities in northern and southern Benin. The trial was a split-plot experiment, where treatments included volume of irrigation water (V1 and V2 = 0.5 *V1) as the whole-plot factor, with organic amendment (45 t/ha RCW; 15 t/ha poultry manure, PM; 45 t/ha RCW + 15 t/ha of PM, RCW_PM; and control) as the sub-plot factor. Topsoil was sampled before and after 24 months of in situ organic amendment application. Humic acid (HA) was extracted from soil and purified prior to FTIR spectroscopy, NMR, and Py-GC-MS analyses. RCW treatments (RCW and RCW_PM) contributed substantially to organic carbon storage and soil nutrient (N, P, K, Ca, Mg) increases (the latter by up to 1.5 times) compared to the control. Accordingly, soils that were amended with the RCW produced significant amounts of HA. High concentrations of lignin-related compounds were determined in pyrolysis products from humic acids in the RCW-amended soils. Using ITS2 region sequencing on the Illumina Miseq platform, RCW amendments markedly altered fungal compositions, with increased relative abundance of Ascomycota (up to 92% of total fungal community), Mucoromycota (2.7%), Basidiomycota (2.5%) and Rozellomycota (0.3%). These fungal phyla contain species that participate in organic matter decomposition and compound transformations but did not selectively degrade lignin in RCW. Our results suggest that RCW amendments significantly increased fungal activity and improved soil nutrient availability. Thus, this agrotechnology is recommended for regenerative agriculture.

1. Introduction

In sub-Saharan Africa, a major challenge for agriculture is finding a sustainable solution to declining soil fertility. Maintaining fertility is important for ensuring food security for the region's growing population. Soil fertility decline in tropical regions is related mainly to low organic matter content (< 1.5%) (Li et al., 2018) and to poor agricultural practices. The latter includes reliance on synthetic fertilizers, mono-cropping, and slash-and-burn practices (Baah-Ofori and Amoakohene, 2021; Manlay et al., 2007). Therefore, there is an urgent need to promote agricultural practices that would sustainably increase soil fertility and soil organic carbon (SOC) stocks to overcome low organic matter levels and mitigate climate change (Chen et al., 2018; Fujisaki et al., 2018). Organic amendments can decrease bulk density, while increasing soil organic carbon, soil porosity, soil water-holding capacity, and water infiltration and percolation (Li et al., 2019; Thangarajan et al., 2013). In addition, organic amendments stimulate soil microbial activity primarily in degraded soils, resulting in the mineralization of organic matter, improvement of soil fertility and then, increase crop yields (Chen et al., 2018). In vegetable crops, the main organic amendments are animal manure (e.g., poultry manure, cow dung, pig dung) and ligno-cellulosic residues (e.g., crop residues, ramial chipped wood) (Thangarajan et al., 2013). Poultry manure (PM) is considered the famous popular organic amendment in vegetable production and has a helpful advantage to the soil quality improvement (Agbede and Oyewumi, 2022; Assogba-Komlan et al., 2007). PM refers to an organic amendment that recycles organic matter and nutrients from manure and is useful for plant growth because of its high macro and micronutrient content (Majeed M. Ali Jaaf et al., 2022). Previous studies revealed that application of PM enhanced soil organic matter and plant nutrients

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which positively increased soil physical and chemical properties, soil water dynamics and, crop yields (Adekiya et al., 2019; Agbede and Oyewumi, 2022). These studies indicated low C/N ratio (an average of 8) and high nutrient contents of PM which allowed decomposition and nutrient release. Bohara et al. (2019) revealed that PM application in soil along with cover crops and conservation tillage preserved soil moisture and enhance soil water retention. However, its beneficial effects is for short duration due to its rapid decomposition (Abbasi and Anwar, 2015) and it may need to be combined with others lignocellulosic biomass to increase its soil residence time. In addition, its wide application, overuse and mismanagement could cause environmental problems due to mineralization of nitrogen, ammonia volatilization and leaching of nutrients (Majeed M. Ali Jaaf et al., 2022). Ramial chipped wood (RCW) applications to soil are useful amendment practices with a high potential for improving soil fertility and soil carbon storage (Barthès et al., 2015; Soumare et al., 2002). RCWs are fragmented (chipped) leafless branches (diameter < 7 cm) of shrubs or trees (preferably, hardwoods), which are used as soil organic amendments (Daassi et al., 2020; Lemieux, 1986). Several studies have reported that amounts of RCW applied to the soil varied by region and soil texture. The review by Barthès et al. (2010) found that depending on biomass availability, 12.5–25 $Mg.ha^{-1}.year^{-1}$ DM of RCW are applied in temperate area on sandy loam soil whereas in subhumid tropical zone 10–30 $Mg.ha^{-1}.year^{-1}$ DM are applied on sandy clay soil. Previous studies have revealed that RCW applications improve soil physicochemical properties by increasing concentrations of soil macro- (N, P, K, Ca, Mg) and micro-nutrients (Fe, Mn, Zn) (Barthès et al., 2010; Daassi et al., 2020; Dhaliwal et al., 2019). Efficient phytoremediation, weed control, and improved crop productivity also have been reported in both long- and short-term RCW amendment studies (Félix et al., 2018; Hattab et al., 2014; Soumare et al., 2002). Given that they are biologically and chemically recalcitrant carbon materials (Daassi et al., 2021), RCWs may enhance recalcitrant carbon pools and physical protection of SOC, which reduces its decomposition and promotes SOC stabilization (Gul et al., 2015; Paul, 2016). Despite these positive effects of RCWs on soil, little is known about how soil moisture might regulate their effect on soil properties, as well as the mechanisms underlying their action, particularly their effects on soil fungal communities and the contributions of RCW lignin to humus formation, which are the basis for good soil health (Chesworth, 2008; Hargitai, 1993).

Soil humus is defined as a complex, heterogeneous and chemically resistant mixture of dark brown or brown amorphous, colloidal substances that are synthesized in soils, sediments, and natural waters by biochemical and chemical reactions during the decay and transformation of plant and microbial remains (Aquino et al., 2019; Bollag and Loll, 1983). Soil humus is considered as a source of progressive nutrient releases and is the major persistent pool of organic carbon (up to 60–80%) in the soil with mean residence time of several hundreds of years (Chesworth, 2008; Stevenson, 1994), with important roles in soil structure and aggregation, regulation of plant nutrition mechanisms, and soil water-holding capacity (Chesworth, 2008; Hayes and Swift, 1990). As components of humus, humic substances (HS) are classified based upon their fractionation into humic acid (HA) (alkali-soluble), fulvic acid (FA) and humin (insoluble residue) (Chesworth, 2008; Hayes and Swift, 1990). HS contains major functional groups, such as phenolic, carboxylic, carbonyl, hydroxyl, amine and amide groups, among others (Chesworth, 2008; Hayes and Swift, 1990; Hernández et al., 2019). Previous studies reported that application of organic amendment influenced soil humus composition and could promote storage of SOC pool (Mi et al., 2019; Zhang et al., 2019). Mi et al. (2019) showed that application of organic amendment (cattle manure and rice straw) increased soil humus carbon which is regarded as an effective indicator of the C sequestration process in the soil. Zhang et al. (2019) found that mineral fertilizer with maize straw or biochar increased soil HA carbon content. Hattab et al. (2014) revealed that application of lignocellulosic amendment significantly increased turnover of C into HA than into FA and humin in soil. It has been estimated that more than 55% of total soil organic matter (SOM) can be extracted during HA fractionation (Chesworth, 2008; Hayes and Swift, 1990). Therefore, these biogeochemical molecules provide essential information regarding the origin and transformation processes of the entire SOM pool. Therefore, HA is often used to represent and characterize the HS (Hernández et al., 2019). The formation, transformation and fate of soil HA are complex processes involving a group of factors, including pH, microbial community composition and activity, environmental hydrophobicity, and climate (Monda et al., 2018; Piccolo et al., 2005). HAs have been previously demonstrated as having a heterogeneous structure that originates in part from microbial reworking of plant bio-macromolecules, largely those of lignin (Chesworth, 2008; Kogel-Knabner, 2002). Research that has been devoted to the study of HA has employed ultraviolet-visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), cross-polarization, magic-angle spinning nuclear magnetic resonance spectroscopy (^{13}C-CPMAS NMR), and pyrolytic analyses. These analyses have revealed important effects of agricultural practices, such as organic amendments, tillage or cropping systems, on the chemical and structural properties of HAs (Aquino et al., 2019; Bai et al., 2015; Wei et al., 2020).

Soil microorganisms play an important role in ligno-cellulosic biomass decomposition and nutrient cycling, and are sensitive to environmental changes (Chen et al., 2018). Most fungal communities are reported to contribute to mobilization and assimilation of soil nutrients, thereby facilitating plant growth and fitness (Ezeokoli et al., 2020). Similarly, fungi play roles in plant pathogen control and soil structure improvement (Bello et al., 2021; Wang et al., 2020). The Basidiomycota, particularly the white-rot fungi, have been shown to facilitate the decomposition of ligno-cellulosic biomass in the soil by producing extracellular heme-peroxidases (lignin peroxidase, manganese peroxidase, versatile peroxidase) and laccase (Datta et al., 2017). Despite all of the research that has been conducted on RCWs, little has been investigated regarding the effects of RCW applications on the composition and diversity of the soil fungal communities and on the structure of humic acids in sub-Saharan Africa.

In the present study, we investigated the effects of RCW amendments that were derived from *Gmelina arborea* and *Sarcocephalus latifolius* and poultry manure in southern and northern Benin ecozones with contrasting climates on (i) soil chemical proprieties and soil carbon storage, (ii) soil humic acid composition, and (iii) soil fungal community composition and diversity.

2. Material and Methods

2.1. Experimental sites and soil types

The experiment was conducted in Benin for two years on two sites with contrasting climates. The two sites are located at the Mèdji Agricultural High School in Sékou (06°41’64" N, 02°34’82" E; Atlantic coastal Benin) and at the Kika Agricultural Technical High School (09°17’09" N, 2°45’06" E; Northeastern Benin), respectively. The Sékou site was characterized by a mean annual temperature of 27.7 °C and 1400 mm of precipitation, while at Kika site, mean annual temperature was 28.9 °C and precipitation was 1200 mm (Daassi et al., 2021). The experiment was conducted from June 2018 to October 2020. Soils of the Sékou site are deep, ferrallitic red soils, which are classified as Ferralsols (World Reference Base for soil resources) with a near-null slope, whose profile showed the colors dark reddish brown (5YR2/3), dark reddish brown (5YR3/4) and red (2.5YR4/8) at the 0–10, 0–20 and 20–100 cm horizons respectively. The crop history of these soils is mainly vegetable production. As for the Kika site, the soils are medium deep indurated tropical ferruginous types on granite-gneiss, which are classified as Luvisols (IUSS Working Group WRB, 2015) with a near-zero slope, presenting the colors dark brown (10YR3/3), pale brown (10YR6/3) and reddish brown (2.5YR4/3) respectively at the 0–10, 10–20 and 20–35 cm horizons. The crop history of the Kika soils is mainly cotton production. Major properties of these soils prior to the experiments are presented in Table 1.

2.2. Experimental design, soil sampling and analysis

The trial was a split-plot layout with four blocks (Fig. 1), which were used in another trial to evaluate agronomic responses of tomato and okra crops to amendments.

The whole-plot treatments were water irrigation volumes with two levels: (i) V1, which is the water irrigation requirement of the crop; and (ii) V2 = ½V1. Weather stations were installed on the experimental sites and rainfall data were collected, allowing adjustment of the watering volume in case of rain shortages. The sub-plots were organic amendments with four levels: (i) 45 $Mg.ha^{-1}$ dry-matter (DM) of ramial chipped wood (RCW) which correspond to 22.5 $Mg.ha^{-1}$ equivalent C; (ii) 15 $Mg.ha^{-1}$ DM of poultry manure (PM) which correspond to 4.8 $Mg.ha^{-1}$ equivalent C; (iii) RCW (45 $Mg.ha^{-1}$ DM) + PM (15 $Mg.ha^{-1}$ DM) mixture (RCW_PM); and control. The eight treatments that were tested were crossed levels of the two factors. RCW treatments were applied to soil so that 50% was buried at 10 cm depth in the soil and 50% was applied as mulch.

The treatment for the organic amendment groups and their applied dose was based upon local farming practices and published papers (Assogba-Komlan et al., 2007; Barthès et al., 2010). These doses are the sum of three application rates of the amendment groups that were applied in a single application package at the beginning of the trial. RCW treatments at Kika only consisted of *Sarcocephalus latifolius* (Sm.) E.A. Bruce (Rubiaceae), which is known commonly as African peach or p ê cher africain. Those at Sékou consisted only of *Gmelina arborea* Roxb. (Lamiaceae), which is also known as white teak or Malay beechwood. One should note that the PM in the case of our study is poultry litter which is a mixture of poultry droppings with sawdust and wood chips, commonly used in field practices in Benin. Initial chemical compositions of RCW derived from *G. arborea* and *S. latifolius* and poultry manure are presented in Table 2.

Composite soil samples were collected prior the trial and 24 months following application of organic amendments at 0–20 cm depth on the experimental sites at Sékou and Kika; their physico-chemical analyses were conducted according to methodologies described by Mathieu and Pieltain (2003). Soil samples were air-dried, ground, and sieved to 0.2 mm for assessing C stocks according to IPCC guidelines (IPCC et al., 2006). Soil particle size determinations were performed using the Robinson pipette method (Robinson, 1949). Bulk pH was measured by potentiometric method using a 1/2.5 soil/water ratio (AB15 pH meter, Accumet Basic, Fisher Scientific). Bulk density (BD) was determined by the core method using 100 cm^3 stainless steel cylinders (Blake and Hartge, 1986). Soil organic carbon (SOC) content was quantified by dichromate oxidation (Walkley and Black, 1934).

Total soil nitrogen (N) content was determined following sulfuric acid digestion, by distillation and titration using the Kjeldahl method (Kjeldahl, 1883). Soil assimilable phosphorus content was determined using an ammonium fluoride extractant (Bray 1), according to the method of Bray and Kurtz (1945). Soil cation exchange capacity (CEC) was determined by the Metson method (Metson, 1956), and soil exchangeable cations (K, Ca and Mg) by atomic absorption spectrometry using Helmke and Sparks' method (Helmke and Sparks, 1996).

SOC stocks were calculated by using Eq. (1) of Ellert and Bettany (Ellert and Bettany, 1995):

$$\text{Stock.SOC}\ (\text{Mg}\ \ \text{ha}^{-1}) = \text{Cont.SOC}\left(\text{g}\ \ \text{kg}^{-1}\right) \text{x BD}\left(\text{g cm}^{-3}\ \ \right) \text{x T (dm)} \tag{1}$$

where Stock.SOC = Soil organic carbon stock (Mg ha^{-1}), Cont.SOC = soil organic carbon concentration (g kg^{-1}), BD = dry bulk density (g cm^{-3}), T = soil layer thickness (dm).

2.3. Humic acid extraction and analysis

Humic acid (HA) was extracted and purified according to the method of International Humic Substances Society (2014). Briefly, dried soil samples were first rinsed with 0.5 M HCl in a ratio of 10:1 (w/v) and centrifuged for 15 min (4 °C, 9000 rpm). The soil residue was mixed with 0.5 M NaOH under nitrogen, and the mixture was stirred for 18 h before centrifugation to separate the alkaline extract from the insoluble extraction residue (humin). The alkaline extract was acidified (6 M HCl) and centrifuged for 15 min (4 °C, 9000 rpm) to separate HA from fulvic acid (FA); the HA fraction was redissolved in aqueous 0.1 M NaOH. The HA fraction was dialyzed against distilled water on the dialysis membrane (MWCO 1000, Spectra/Por® 7 dialysis tube) until the water was colourless outside of the dialysis tube (Bai et al., 2015; Garciá et al., 2016; Wei et al., 2020). At the end of the dialysis step, the HA fraction was lyophilized before analyzing its properties. The elemental composition (C, H, N and O) was determined using a Perkin Elmer 2400 CHN (Norwalk, CT, USA) elemental analyzer. Ash content of HA was determined using the ASTM standard method, which is described by the International Humic Substances Society (2014). HA fraction was analyzed using scanning ultraviolet-visible (UV-Vis) spectroscopy recording the spectra between 465 and 665 nm, with 5 nm per scanning step increment. The E6/E4 ratio of HA was determined by dividing absorbance that was measured at 665 nm by that at 465 nm (Bento et al., 2020). Fourier-transform infrared (FTIR) spectra of HA were acquired from 4000 to 650 cm^{-1} with 64 scans per sample at a resolution of 4 cm^{-1} using a PerkinElmer Spectrum 400 (Monda et al., 2018). Cross-polarization magic-angle spinning (CPMAS) carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on the HA fraction with a Varian NMR spectrometer at 500 MHz (Aquino et al., 2019; Monda et al., 2018; Xu et al., 2019). Spectra were acquired by using 2 s of recycle delay followed by 1 ms of contact time, 30 ms of acquisition time and 4000 scans. Based on previous work, five chemical shift regions were assigned to the major organic functional groups: 160–190 ppm (carboxyl-C), 145–160 ppm (O-aryl-C), 110– 145 ppm (aromatic-C), 60–110 ppm (O-alkyl-C), 45–60 ppm (O-substituted alkyl-C), and 0–45 ppm (aliphatic-C) (Aquino et al., 2019; Monda et al., 2018, 2017). HA fraction was analyzed in duplicate by Py-GC/MS in the presence of tetra-methyl ammonium hydroxide (TMAH) using a probe pyrolyzer (CDS Pyroprobe 2000) and a gas chromatograph (CP 3800, Varian), which was coupled to a mass spectrometer (Varian Saturn 2200, 30–650 u.m.a). Briefly, 0.4 mg of HA was impregnated with 10 μL of TMAH in 25% methanol prior to pyrolysis. The injection temperature of the GC (rate of 20 °C/s) and the GC/MS interface were set at 250 °C and

Table 1
Initial soil physico-chemical parameters at 0–20 cm depth at Kika and Sékou sites.

Soil parameters	Kika	Sékou
pH_{H2O}	6.66 ± 0.43	6 ± 0.37
BD[a] (g cm^{-3})	1.34 ± 0.05	1.24 ± 0.08
SOC (g kg^{-1})	10.04 ± 0.4	5.24 ± 0.93
N (g kg^{-1})	1.15 ± 0.08	0.94 ± 0.13
SOC Stock (Mg ha^{-1})	27.02 ± 1.63	19.65 ± 4.28
P (mg kg^{-1})	11.82 ± 0.46	11.78 ± 0.37
C/N	8.87 ± 0.71	6.1 ± 1.64
CEC (cmol kg^{-1})	19.17 ± 1.57	12.5 ± 0.87
K^+ (cmol kg^{-1})	0.53 ± 0.14	0.22 ± 0.08
Ca^{+2} (cmol kg^{-1})	4.63 ± 1.34	4.64 ± 0.53
Mg^{2+} (cmol kg^{-1})	1.93 ± 0.5	1.82 ± 0.41
Clay (%)	8 ± 0.3	11.9 ± 1.4
Silt (%)	13.3 ± 1	12.2 ± 2
Sand (%)	78.3 ± 0.9	75.3 ± 1.7
Soil texture	Sandy loam	

[a] Bulk density; values of soil parameters are expressed as means ± standard errors, n = 3. SOC and N are quantified in total form, while P in labile form and, Ca, K, Mg are in exchangeable form.

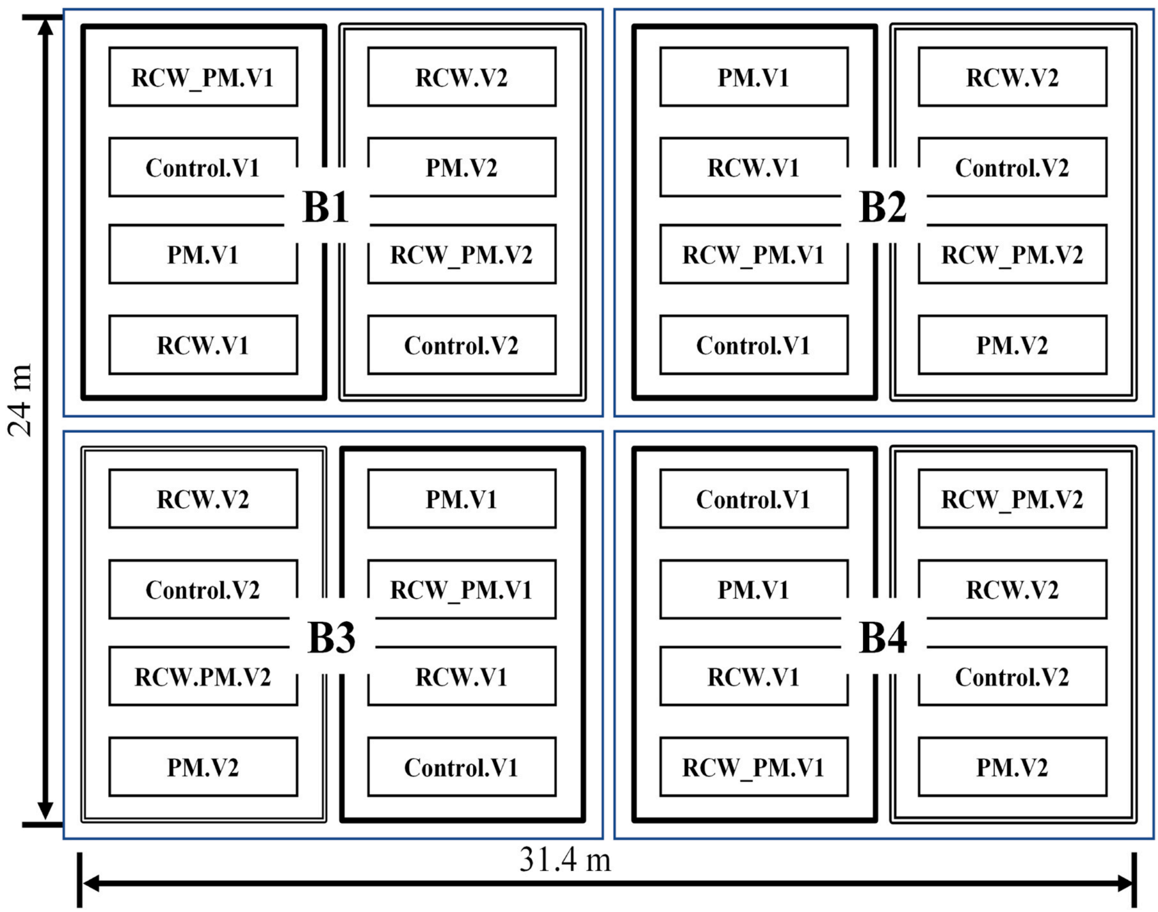

Fig. 1. Experimental layout in split-plot design. RCW: ramial chipped wood, PM: poultry manure, V: water irrigation volume.

Table 2
Initial chemical and mineral compositions of ramial chipped wood and poultry manure (Daassi et al., 2020).

	Poultry manure	RCW from *G. arborea*	RCW from *S. latifolius*
pH	7.73 ± 0.1	5.0 ± 0.0	6.5 ± 0.0
C (g.kg^{-1})	322.1 ± 9.1	499.1 ± 1	500.5 ± 11
N (g.kg^{-1})	21.0 ± 1.0	5.0 ± 1.0	6.0 ± 0.0
C/N	15.34 ± 2.1	94.9 ± 5.2	82.8 ± 0.5
P (mg.kg^{-1})	5021.8 ± 89.1	537.2 ± 4.0	1061.9 ± 18.9
K (mg.kg^{-1})	8733.6 ± 23.2	5149.1 ± 36.1	8852.6 ± 14.4
Ca (mg.kg^{-1})	9123.3 ± 76.2	6059.9 ± 30.7	7110.8 ± 61.9
Mg (mg.kg^{-1})	4831.3 ± 94.2	2150.9 ± 27.7	3360.1 ± 36.3
Fe (mg.kg^{-1})	931.1 ± 72.1	94.5 ± 4.1	1040.4 ± 33.1
Zn (mg.kg^{-1})	93.4 ± 5.2	16.1 ± 1.2	13.7 ± 0.4
Mn (mg.kg^{-1})	210.0 ± 2.1	61.8 ± 0.7	59.7 ± 1.2

Chemical compositions are expressed as means ± standard errors, n = 3. All elements that were determined (C, N, P, K, Ca, Mg, Fe, Zn and Mn) are quantified in their total form.

maintained for 10 s, with He used as the carrier gas. The mass spectrometer was scanning in the range of $m/z = 35$–400. Pyrolysis products were identified using the NIST (Gaithersburg, MD, USA) mass spectral library and completed using available published work (Aquino et al., 2019; Klap et al., 1998; Savarese et al., 2021).

2.4. Soil DNA extraction, Illumina MiSeq and bioinformatic analysis

Total DNA was extracted by processing 50 mg of soil samples using the Power Soil DNA Isolation Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The libraries were prepared using a two-step dual-indexed Polymerase chain reaction (PCR) approach, which was specifically created for Illumina instruments based on primer biases in fungal meta-barcoding (Tedersoo et al., 2015). Amplification of the fungal ITS2 region was performed on a 50 μL PCR reaction mixture composed of 40 μg of DNA, 0.2 μM of dNTPs, 0.2 μM of each primer, 1X of buffer and amplifier supplied by New England Biolabs (Ipswich, MA, USA) and 1.25 μM of TopTaq DNA polymerase. The first and second PCR reactions were performed in a thermal cycler and purified using an Axygen Biosciences (Axygen Biosciences, 2013) magnetic stir plate with magnetic beads in ultra-pure water and 80% ethanol at a ratio of 0.9/1. Amplicons that were pooled in equimolar concentrations were sequenced by illumina Miseq at the genomic analysis core facility (IBIS, Université Laval, Quebec). After sequencing, all raw FASTQ data were processed using the Quantitative Insights Into Microbial Ecology (QIIME 2 2020.2.0, https://qiime2.org) pipeline (Bolyen et al., 2019). The DADA2 denoiser was used to obtain, from the paired-end sequences, the amplicon sequence variants (ASVs) according to their quality ($Q > 25$) (Callahan et al., 2016). Taxonomic assignment of ASVs was performed against the UNITE-ver7–99 reference database (Abarenkov et al., 2020) using the fit-classifier-naive-bayes plugin of the QIIME2 feature classifier at a confidence threshold of 99% operational taxonomic units (OTUs) (Rognes et al., 2016). The OTU tables were rarefied to even depths of 10000 ASVs before obtaining alpha and beta diversities in QIIME2, which were followed by statistical analyses.

2.5. Statistical analyses

Statistical analyses were performed in R V4.1.1 (R Core Team, 2017) and SAS V9.4 (SAS Institute Inc. 2013). The influence of soil chemical properties on fungal community composition and structure in the Sékou and Kika sites was estimated by canonical correspondence analysis (CCA) and by Spearman rank and heat map correlations (r_s). CCA was performed on the log-transformed soil chemical parameters and fungal community data by using the "ord" function in package *vegan* (Oksanen et al., 2019). The significance of these chemical variables (constraining) was tested with "envfit" based on 999 permutations in R. Multi-collinear constraining variables (with variance inflation factors > 10) were excluded from the final CCA bi-plot.

Fungal OTU relative counts were determined after $\log_{10}$ transformation using the "decostand ()" function (*vegan* package) of R software, as described by Oksanen et al. (2019). The significant effects of organic amendments and water irrigation on alpha diversity indices (Shannon index, Pielou Evenness, OTU richness, and Faith phylogenetic diversity index), soil chemical parameters and characteristic indices of humic acid were examined using analyse of variance (ANOVA) with the mixed-model procedure of SAS (SAS Institute Inc. 2013). Normality and homogeneity assumptions were confirmed and the differences between treatments were tested using Tukey HSD tests at $P < 0.05$.

3. Results and Discussion

3.1. Dynamics of soil -chemical parameters

The ANOVA performed with the mixed-model procedure showed that organic amendments significantly changed most of soil chemical parameters (pH, SOC, N, P, CEC, K, Ca and Mg) at 0–20 cm depth under the influence of water irrigation volume at the Kika and Sékou sites (Fig. 2 and Fig. 3). Soil acidity decreased when the RCW and PM mixture was applied compared to the single RCW and to initial soil acidity prior to amendment (Table 1); the decrease was more pronounced in Sékou soil (Fig. 3A) than in Kika (Fig. 2A).

Organic amendments (compost, ligno-cellulosic residues, poultry manure, biochar) are known to exert a buffering effect regulating or correcting soil acidity that favours soil microbial activity and effective plant growth (Manlay et al., 2007). This response could explain the decrease in acidity of the amended soils in this study. Bulk density did not vary between organic treatments in the Kika and Sekou soils, which can be explained by the relatively short duration (24 months) of the experiment. This meant that applied amendments did not affect soil physical parameters, such as bulk density and soil texture, which

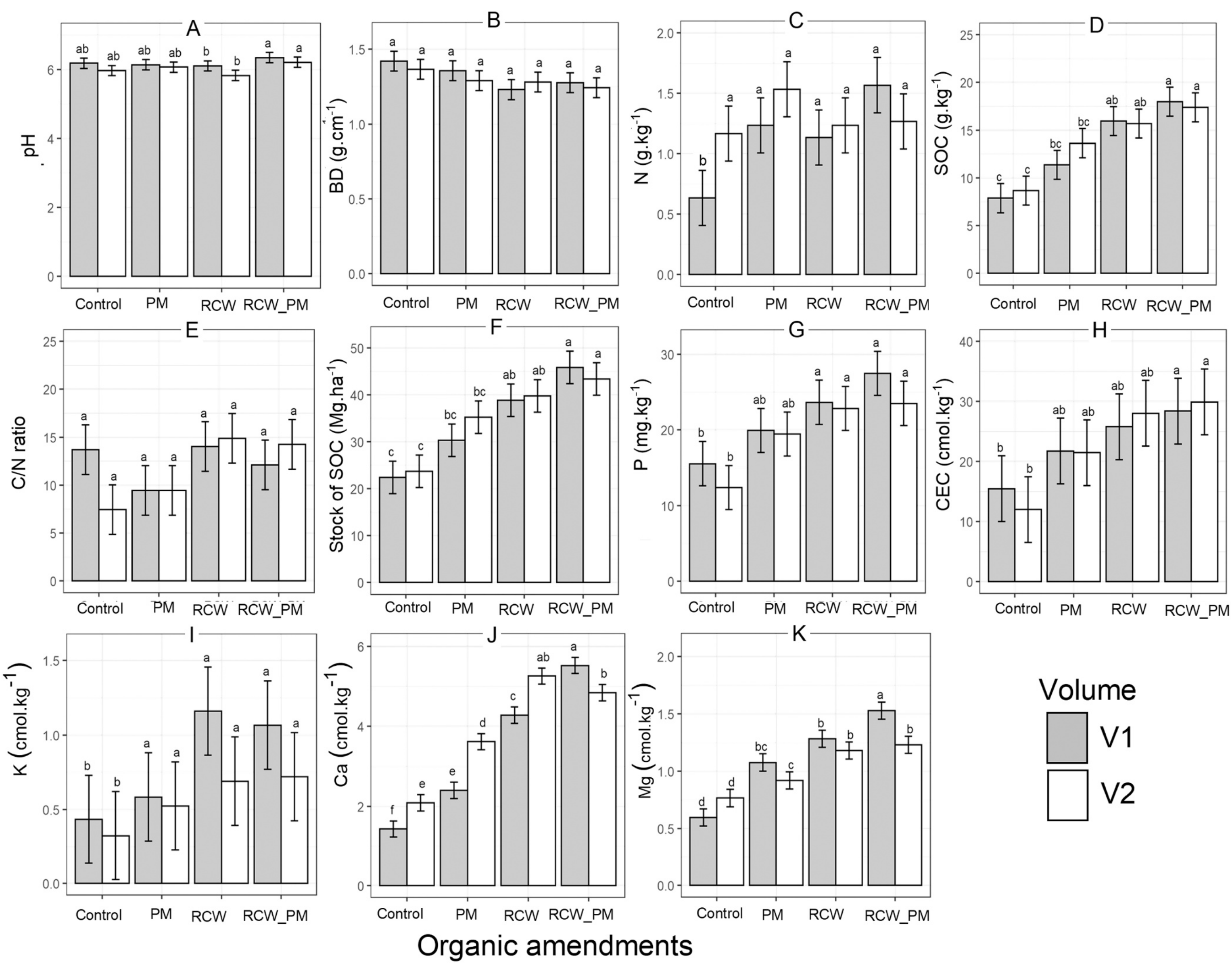

Fig. 2. Soil chemical properties after two years of organic amendments at Kika. RCW: ramial chipped wood, PM: poultry manure, RCW of *S. latifolius* were applied in Kika site, respectively. V1: water requirement for plant irrigation, V2: 1/2V1, Means are followed by standard errors. Means with the same letter do not significantly differ (Tukey HSD tests, $P < 5\%$). In Fig. 2, the letters A, B, C, D, E, F, G, H, I, J and K represent respectively pH, BD, N, SOC, C/N ratio, Stock of SOC, P, CEC, K, Ca and Mg.

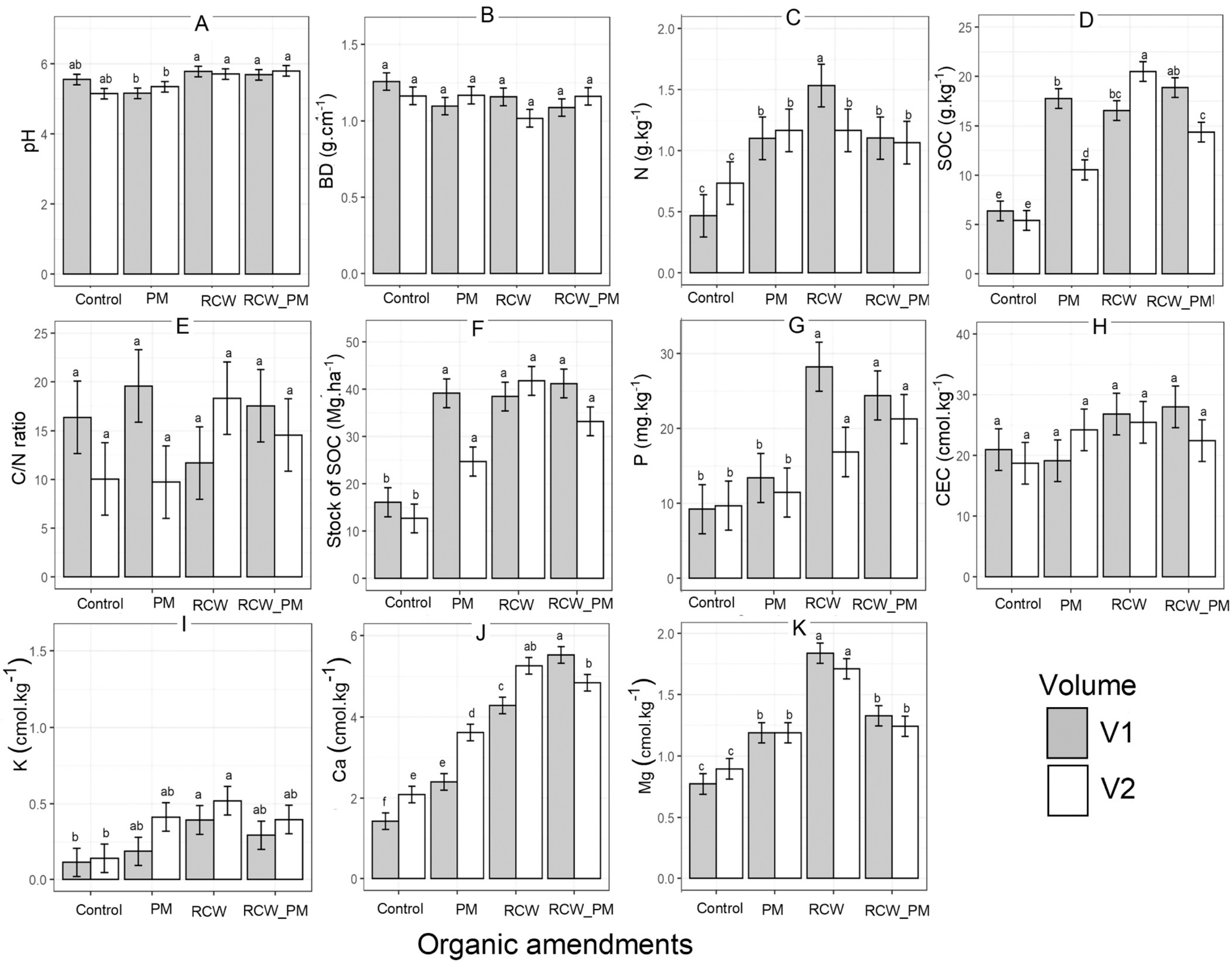

Fig. 3. Soil chemical properties after two years of organic amendments at Sékou. RCW: ramial chipped wood, PM: poultry manure, *G. arborea* RCWs were applied in Sékou site. V1: water requirement for plant irrigation, V2: 1/2V1, Means are followed by standard errors. Means with the same letter do not significantly different (Tukey HSD tests, $P < 5\%$). In Fig. 3, the letters A, B, C, D, E, F, G, H, I, J and K represent respectively pH, BD, N, SOC, C/N ratio, Stock of SOC, P, CEC, K, Ca and Mg.

remained sandy loam (Table 1). These findings are supported by the results of other published studies (Bello et al., 2021; Ellert and Bettany, 1995; Rehrah et al., 2016). Significantly higher concentrations of N, P, soil organic carbon (SOC), and stocks of SOC ($P < 0.05$) were detected for Kika and Sékou soil plots that were amended with RCWs (RCW and RCW_PM) compared to those amended with PM, the control plots and the initial state of the soil (Fig. 2 and Fig. 3). Several studies on RCW experiments in temperate and tropical regions have reported an increase in SOC. N'dayegamiye and Dubé (1986) noted a significant increase of SOC (20–70% compared to control) after RCW soil amendment. They recognized that the combined application of pig manure and woody matter promotes the decomposition and humification of RCW on sandy gravel soils in Quebec. Other temperate zone studies reported that for the second year of the RCW experiment, there was a significant increase in SOC content (> 20%) in sandy loam soils (Cardinael et al., 2017; Scharenbroch and Watson, 2014; Tremblay and Beauchamp, 1998). Yet, few studies in tropical regions have reported results as conclusive as those that have been reported in the temperate region. Soumare et al. (2002) observed that RCW incorporation into soils had no effect on soil organic carbon relative to the control. In their experiment, the authors noted that RCWs, which had been applied in a fresh state, had not been sufficiently decomposed by the end of the trial, resulting in limited effects on organic carbon levels. Similar findings were reported for a Sudano-Sahelian ecosystem, where no significant increase of organic carbon was found after application of RCW in sandy soil compared to the control (Barthès et al., 2015; Félix et al., 2018). This was explained by the priming effect and by weak protection of organic matter against mineralization (Fontaine et al., 2003; Manlay et al., 2007). Contrary to the results of those studies, our results showed a significant increase in organic carbon after RCW-based treatments were applied to sandy loam soil (Table 1) at the Kika and Sékou sites. This can be explained by the relatively high rate of decomposition for the RCWs that were applied in our study, as observed from the low percentage of RCW mass-remaining after 24 months (Daassi et al., 2021). Our findings are corroborated by other RCW studies in sub-Saharan Africa (Félix et al., 2018; Kerrouche et al., 2020). Soil organic carbon (SOC) stocks varied significantly among treatments. Initial SOC stocks prior to the experiments were 27.02 ± 1.63 Mg ha^{-1} at Kika and 19.65 ± 4.28 Mg ha^{-1} at Sékou site (Table 1). These values are close to those that were reported from studies performed in the same ecozones (Koussihouèdé et al., 2017; Volkoff et al., 1999). At the Kika site where we had applied the RCW of *S. latifolius*, SOC stocks under RCW_PM (45.83 ± 3.65 Mg ha^{-1}) were significantly higher ($P < 0.01$) than those in the controls (22.37 ± 3.65 Mg ha^{-1}) and for the initial state of the soil (27.02 ± 1.63 Mg ha^{-1}) at 0–20 cm depth. Similarly, SOC stocks were significantly higher under RCW plots (41.76 ± 3.18 Mg ha^{-1}) than that in control plots (12.68

± 3.18 Mg ha^{-1}) and the initial state of the soil (19.65 ± 4.28 Mg ha^{-1}) at the Sékou site. At both sites, the decrease in SOC stocks determined for the control treatment compared to the initial soil values could be explained by the management of the treated plots during the trial. Indeed, three successive productions of tomato and okra were carried out on the amended soils, which lasted about 24 months (data not shown). The light tillage performed to maintain the amended soil beds during production of these crops appears to promote relative soil compaction, which induced a slight increase in bulk density of the control treatment relative to the initial soil (Table 1, Fig. 2B, Fig. 3B). Likewise, no crop residues except roots (which were naturally incorporated) were returned to the treated soils after crop production. This indicates that no organic inputs were induced on the control treatments following crop production. This is in accordance with other studies which demonstrated that the decline of organic matter is caused by several factors, such as climate, texture, hydrology, land use and vegetation (Chen et al., 2021; Söderström et al., 2014). In the Sékou site, we used RCW that was derived from *G. arborea*. The SOC stock that was calculated for RCW-based treatments of soil at both sites is close to the estimates (42 Mg ha^{-1}) that were obtained by Barthès et al. (2004) and Aholoukpè et al. (2016). These authors obtained these estimates after 10 years of incorporation of mucuna (*Mucuna pruriens* [L.] D.C., Fabaceae) residues in soil in a maize-mucuna system, and in a system of total incorporation to the soil of pruned fronds in a palm oil (*Elaeis guineensis* Jacq.) plantation on a Ferralsol in Benin, respectively.

The cited findings confirm that crop residues or woody biomass applications promote soil carbon sequestration, including the system that was based upon RCW amendments. In our study, the application of RCW alone, or combined with PM, contributed to almost a doubling of the SOC stock 24 months after application, thereby indicating substantial storage of carbon in soil following RCW treatment. The increase in SOC stocks after application of ligno-cellulosic biomass to soils is beneficial not only for mitigating climate change, but also for improving soil quality and fertility, with major potential positive effects on crop yields (Chen et al., 2019; Eze et al., 2018; Lorenz et al., 2007). In addition, substantial increases in N, P, K, Ca, Mg and cation exchange capacity (CEC) of the soils following RCW-based treatments (compared to the control) corroborated the results of other published studies (Chen et al., 2018; Félix et al., 2018; Soumare et al., 2002). These results represent a good indicator of improved soil fertility following the RCW application. In addition, our results showed that irrigation water volume had a significant effect only on exchangeable bases (Ca, Mg) at the Kika site (Fig. 2), while it influenced only SOC, Ca contents and SOC stocks at the Sekou site (Fig. 3). Since two volume levels (V1 and V2 =1/2V1) of irrigation were applied to the soil, it affected soil moisture (data not shown), which could probably influence the carbon content of the amended soil. At the Sékou site, the SOC content of the soil under RCW treatment is higher when the soil is less irrigated (below volume V2). This result is contrary to what is reported in the literature. Patel et al. (2021) showed that soil moisture influences soil carbon dynamics, including microbial growth and respiration. They reported that there is a significant disconnection between soil pores under low moisture conditions. Increasing soil moisture can reduce oxygen diffusion in the soil and decrease microbial activity, and thus reduce microbial decomposition of SOC, which is conducive to soil carbon accumulation (Patel et al., 2021; Ren et al., 2022). In the case of the Sékou site, the increase in SOC under low irrigation could be due to the relatively low abundance of decomposers on the RCW treatments (Fig. 5). This could indicate low activity of soil microorganisms involved in RCW decomposition, thus justifying the accumulation of SOC. In addition, Qu et al. (2021) reported that soil moisture strongly influences SOC content and stocks, which are modulated by soil clay content. The soil at the Sékou site is richer in clay (11.9 ± 1.4%) than at the Kika site (8 ± 0.3%), and the differences in chemical and mineral composition between the organic amendments applied at the two sites (Table 2) could also explain the marked variations in Ca, Mg and SOC content and stocks determined at the two experimental sites.

3.2. Effect of organic amendments on humic acids

3.2.1. Yields, E4/E6 ratio and elemental analysis of humic acids

The extraction yields, E4/E6 ratio and elemental composition of humic acids (HA) that were extracted from Sékou and Kika soils are presented in Table 3.

Among the studied parameters, only organic amendment contributed significantly ($P < 0.001$) to variation in HA. Yields of extracted HA varied significantly and were higher in the amended soils (RCW, RCW_PM, PM) than in the control soils for both Sékou and Kika sites. This is likely due to the presence of undecayed ligno-cellulosic residues after amendment, which contributed to the higher yields of humic acid. Consistent with increases in SOC (Fig. 2 and Fig. 3) in the amended soils compared to the control, progressive enrichment of the HA was observed for amended soils in both experimental sites. Our previous work demonstrated that ramial chipped wood (RCW) undergoes extensive decay, but not the lignin therein (Daassi et al., 2021). This process is slower when water is limited or poultry litter with its high N content is not present. Residual lignin from RCW seems to contribute to a large portion of humic acids. Similar enrichment in HA in various organic materials (soil, compost, and biochar, among others) amended soil has been reported in other published work (Bento et al., 2020; Piccolo et al., 2005; Spaccini et al., 2002). Enrichment in SOC following organic amendments is usually attributed to microbial oxidation of decayed organic matter. Our findings suggest that RCW amendments contributed to an increase in soil HA content more than was the case for amendments that were based upon poultry manure, or in control soils. The resulting ash content of all extracted HA was < 6%. The E4/E6 ratio is traditionally used to determine soil humus quality and to deduce the degree of aromaticity and humification. Results presented in Table 3 indicated that the HA extracted from RCW-treated soil had the lowest E4/E6 ratios, followed by those of RCW_PM, PM and control soils. This suggests that the RCW amendments provide the soil with a more condensed aromatic network and contribute to HAs with higher molecular weight compared to PM and mixed RCW_PM amendments. These differences were more prominent for Kika than for Sékou. The observed differences could be related to the quality of RCWs that were applied as amendments in the two experimental sites. As reported in our previous study (Daassi et al., 2020), lignin from *S. latifolius* RCWs that were used at the Kika site was more strongly condensed and, therefore, more recalcitrant to degradation than that derived from *G. arborea* RCW (Sékou site). Quality and quantity of organic amendments are the major factors affecting soil humic acid quality and content (Abril et al., 2013). With respect to elemental compositions of extracted humic acids, the observed values are within the ranges that have been reported for humic acids that have been extracted from other soils (Bento et al., 2020; Monda et al., 2017; Piccolo et al., 2005; Spaccini et al., 2002; Spaccini and Piccolo, 2009). The C-content of HA increased significantly from control (35.4 ± 0.8–36.7 ± 0.7%) soils to soils that were amended with RCW (38.8 ± 0.7–45.3 ± 0.8%) and PM (37.4 ± 0.8–38 ± 0.7%) in Sékou and Kika sites. HA extracted from the RCW-treated soil had the highest N content among HAs that were extracted from organic amendment-treated soil at the Sékou site, while no significant difference was determined in that respect at the Kika site 24 months after amendment. The low N content of HA recorded in PM treatment compared to RCW treatment could be due to the volatilization and leaching of nitrogen during PM decomposition. Moreover, PM being a mineralized fertilizer, did not allow for a long N retention in soil, which could explain the N decrease in HA extracted from soils treated by PM 24 months after amendment at the Sekou site. One should note that nitrogen content determined at 8 and 16 months after amendment (data not shown) revealed that nitrogen content was significantly higher in HAs extracted from PM treated soils than in RCW counterparts or control. At

Table 3
Yield, E4/E6 and elemental composition of humic acids (HA) from Sékou and Kika soil.

	Yield (%)	E4/E6	C (%)	H (%)	O (%)	N (%)	C/N
Kika soil							
Initial	14.2 ± 1.5	4.7 ± 0	37.9 ± 0.6	4.5 ± 0.1	54.2 ± 0.9	2.6 ± 0.1	14.6 ± 0.3
RCW.V1	14.8 ± 1.8 b	2.8 ± 0.1c	39.1 ± 0.7a	4.3 ± 0.1a	53.2 ± 0.8ab	2.7 ± 0.1a	14.5 ± 0.2ab
RCW_PM.V1	14.5 ± 1.8 b	3.2 ± 0.1b	39.9 ± 0.7a	4.1 ± 0.1ab	52.5 ± 0.8b	2.8 ± 0.1a	14.3 ± 0.2a
PM.V1	13.7 ± 1.8bc	3.3 ± 0.1b	38 ± 0.7ab	3.7 ± 0.1b	54.5 ± 0.8ab	2.7 ± 0.1a	14.1 ± 0.2b
Control.V1	12.2 ± 1.8c	4.8 ± 0.1a	36.3 ± 0.7b	4.2 ± 0.1ab	55.8 ± 0.8a	2.8 ± 0.1a	13.0 ± 0.2c
RCW.V2	16.9 ± 1.8 a	2.8 ± 0.1c	38.8 ± 0.7a	4.2 ± 0.1a	54.5 ± 0.8ab	2.8 ± 0.1a	13.9 ± 0.2b
RCW_PM.V2	14.2 ± 1.8 b	3.2 ± 0.1b	39.3 ± 0.7a	4.0 ± 0.1ab	52.8 ± 0.8b	2.7 ± 0.1a	14.6 ± 0.2ab
PM.V2	15 ± 1.8 b	3.4 ± 0.1b	37.5 ± 0.7ab	3.8 ± 0.1b	54.1 ± 0.8ab	2.8 ± 0.1a	13.4 ± 0.2a
Control.V2	11.8 ± 1.8c	5 ± 0.1a	36.7 ± 0.7b	4.1 ± 0.1ab	55.5 ± 0.8a	2.7 ± 0.1a	13.6 ± 0.2c
Sékou soil							
Initial	11.7 ± 2.8	5.6 ± 0.1	39.1 ± 0.9	4.5 ± 0.1	52.3 ± 1.2	2.9 ± 0.1	13.5 ± 0.2
RCW.V1	13 ± 2.2 a	3.3 ± 0.1c	45.3 ± 0.8a	4.5 ± 0.1a	49.1 ± 1b	3.5 ± 0.1a	12.9 ± 0.1a
RCW_PM.V1	12 ± 2.2 ab	3.8 ± 0.1b	41.3 ± 0.8a	4.6 ± 0.1a	49.9 ± 1b	3.2 ± 0.1b	12.9 ± 0.1a
PM.V1	9.3 ± 2.2 b	4.1 ± 0.1b	37.7 ± 0.8b	4.7 ± 0.1b	54.1 ± 1a	2.9 ± 0.1c	13.0 ± 0.1a
Control.V1	9.7 ± 2.2 b	4.8 ± 0.1a	35.8 ± 0.8b	3.9 ± 0.1b	57.6 ± 1a	2.9 ± 0.1bc	12.3 ± 0.1b
RCW.V2	14.7 ± 2.2 a	3.2 ± 0.1c	44.9 ± 0.8a	4.5 ± 0.1a	48.5 ± 1b	3.5 ± 0.1a	11.0 ± 0.1a
RCW_PM.V2	11.2 ± 2.2 ab	3.7 ± 0.1b	41.7 ± 0.8a	4.5 ± 0.1a	49.8 ± 1b	3.0 ± 0.1b	13.9 ± 0.1a
PM.V2	11.1 ± 2.2 ab	3.9 ± 0.1b	37.4 ± 0.8b	4.1 ± 0.1b	54.5 ± 1a	2.8 ± 0.1c	13.4 ± 0.1a
Control.V2	6.9 ± 2.2c	4.7 ± 0.1a	35.4 ± 0.8b	3.8 ± 0.1b	57 ± 1a	2.8 ± 0.1bc	12.6 ± 0.1b

NOTE: Means with the same letters within each column indicate no significant difference among treatments at $P = 0.05$, according to post-hoc Tukey HSD tests.

24 months after amendment, N content in HAs extracted from the PM treated soils decreased significantly, showing the short-term effect of PM and the contribution of RCW to improving nutrient retention, especially nitrogen, as reported in other studies (Agbede and Oyewumi, 2022; Hattab et al., 2014; Robert et al., 2014). The significant differences in the content of H and O, and the C/N ratio of HA were determined between the amended soils. The C/N ratios reflect the variation of C and N content in HA and that in studied soils (Table 1). Thus, higher values of C/N ratios were determined for HAs extracted from amended soils (RCW, RCW_PM and PM) than that extracted from control soils, reflecting the enrichment in soil carbon. The stability of C/N values for HA from amended soils indicates the ability of this fraction to resist microbial decomposition due to their recalcitrant nature (Monda et al., 2018; Spaccini et al., 2013).

3.2.2. FTIR analysis of HA

FTIR spectra of HA were compiled and presented in Fig. 4. They were similar in terms of the positions of major peaks. Several appreciable differences were noted in the intensity of several peaks for HA fractions from amended soils. HA fractions from control and PM samples showed higher absorption signals at 1041 cm^{-1} than for HA extracted from RCW and RCW_PM treated soils at both sites, suggesting that HA from RCW-based treatments had lower carbohydrate content than HA from control and PM samples; the signal at 1041 cm^{-1} is attributed to the C-O asymmetric stretch vibration of carbohydrates (Kalbitz et al., 1999; Monda et al., 2018). Absorption bands at 1213 and 1615 cm^{-1} are attributed to aromatic ethers C–O–C and to aromatic C=C, H-bonded C=O, NH_2 deformation groups, respectively (Kalbitz et al., 1999). These were higher in HA from RCW-based samples than in HA from control samples at Kika. The distinct peak at 2919 cm^{-1} is assigned to C-H

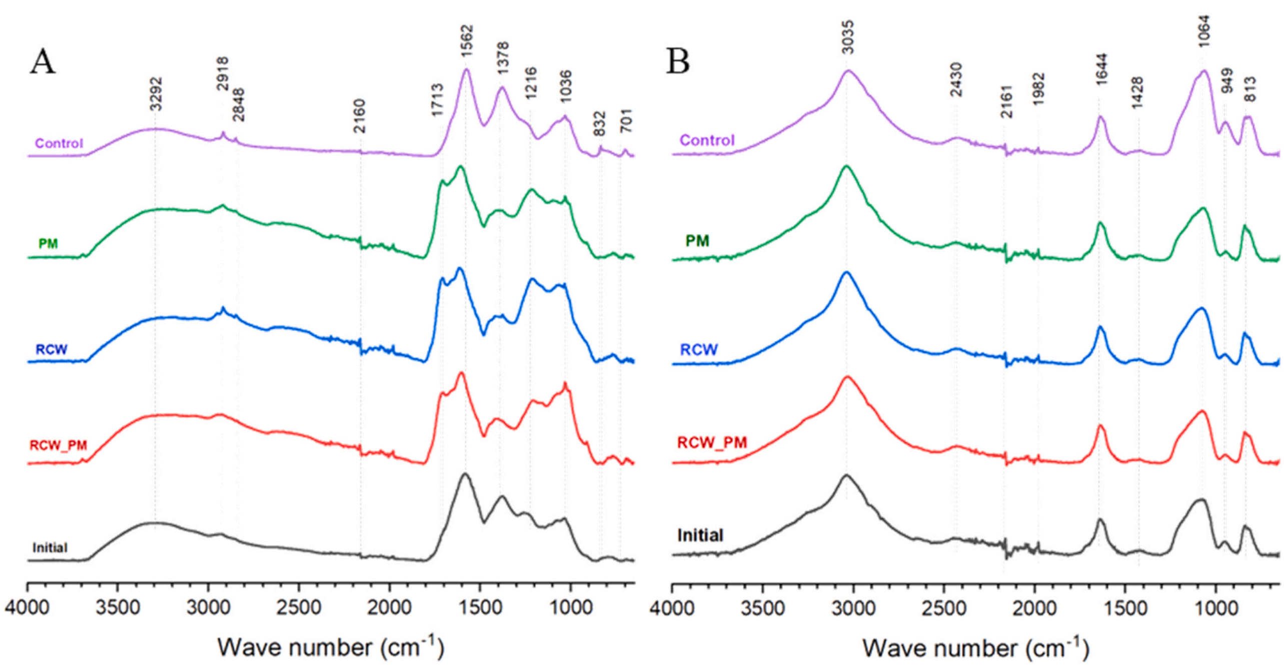

Fig. 4. Fourier-Transform Infrared (FTIR) spectra of humic acids at Kika (A) and Sékou (B) sites for the following treatments: Initial- soil initial state; RCW- ramial chipped wood; PM- poultry manure; RCW_PM- mixture of RCW and PM; control- soil without organic amendment.

stretching of alkyl structures and was higher in HA that was extracted from soils with the RCW treatment, indicating that HA fractions from the Kika site may contain fewer methylene or methyl groups than those of control soil samples.

3.2.3. ^{13}C-CPMAS NMR of HA

The ^{13}C-CPMAS NMR spectra of HA that were extracted from amended soils are shown in Fig. S1, while relative distributions of carbon from main functional groups over chemical shift regions are presented in Table 4.

These spectra revealed the common composition of natural organic biomass with organic carbon distribution across the chemical shift range. Strong peaks in alkyl-C (45–0 ppm), C-O/C-N (60–45 ppm) and O-alkyl-C (110–60 ppm) regions that are shown in the NMR spectra for the HA extracted from all treatments revealed the important presence of alkyl, nitrogen and sugar components, respectively. The broad resonance centred at 29.2 ppm revealed the presence of methyl and methylene groups, presumably from various lipid compounds, bio-polyesters and waxes (Aquino et al., 2019; Monda et al., 2018; Spaccini and Piccolo, 2009); these components were more concentrated in HA that was extracted from control soil of the Sékou and Kika sites. The resonance in the 60–45 ppm region indicated the presence of methoxy groups in lignin structures and nitrogen functions in peptide and amino-acid moieties (Monda et al., 2017; Spaccini and Piccolo, 2009). In particular, the peak at around 56 ppm could be assigned to O-CH_2 and O-CH_3 groups in propylic side-chains of lignin monomers and aromatic rings, respectively (Hatfield et al., 1987; Monda et al., 2018), which were more prominent in HA extracted from RCW-treated soils (R CW and RCW_PM). The intense resonance depicted around 72 ppm (Fig. S1 A and B) indicates the overlapping resonance of C2, C3 and C5 in pyranoside moieties of cellulose and hemicelluloses, while the peak at around 102 ppm (Fig. S1 A and B) corresponds specifically to anomeric C1 of glucose cyclic forms (Aquino et al., 2019; Monda et al., 2018). HAs that were extracted from soils without organic amendments (control and initial treatments) were more concentrated in carbohydrates (O-alkyl-C), accounting for 22.76–37.05%, than those in HAs that were extracted from RCW-treated soils (20.78–26.57%). The results also were confirmed from FTIR results. Conversely, in the aryl region (145–110 ppm), a slight presence of aromatic components was revealed in all HA spectra and was more prominent in soils that were amended with RCW and PM than in control samples. The peaks in the 145–160 ppm broad band correspond to O-substituted-C in aromatic rings of lignin molecules and polyphenol compounds. More specifically, the peaks at around 129–132 ppm are assigned to C3, 4, and 5 in lignin aromatic rings (Monda et al., 2018). These were 3.7 and 3.5 times more concentrated in HA that was extracted from RCW-treated soils than in the control samples for Sékou and Kika soils, respectively. Finally, the signal at around 175 ppm revealed carbonyl-C that was present in various components (fatty acids, esters, amide-C, among others). Structural indices such as aromaticity (ARM), alkyl-C/O/alkyl-C (A/OA), lignin ratio (LigR), and hydrophobicity (HB/HI), which are presented in Table 4, summarize the significant differences in molecular composition between HAs from amended soils. Specifically, ARM, A/OA and HB/HI indices are typically associated with biochemical stability of various organic materials. Indeed, the higher their relative values, the more the organic C stabilization process increases in soil, which is related to the distinct accumulation of hydrophobic compounds (Monda et al., 2017; Piccolo et al., 2005). As for the LigR ratio of C-O/C-N to phenolic C, it shows the relative abundance of lignin structures in HAs that are extracted from amended soils. The most hydrophobic HAs appear to have originated from soils that were amended with RCW_PM and RCW, with the highest ARM indices and HB/BI ratios (Table 4). The highest value of these structural indices suggests a significant contribution of RCW lignin to the formation of HA from amended soil, as has been reported by Monda and coworkers (Monda et al., 2017). In Sékou and Kika soils, the HA that was extracted from soil amended with RCW exhibited a lowest LigR (2.36–3.59) relative to soil without amendment (5.32–12.10), indicating greater preservation and stabilization of lignin residues in corresponding soils.

3.2.4. Py-TMAH GC-MS of HA

Thermochemolysis of HAs released about hundred compounds from HA samples. These could mainly be classified as methyl ethers and esters of pyrolysis products since TMAH was used as a methylating agent (Fig. S2, Table S1). Most of the compounds that had been released were identified as being derived from lignin, along with fatty acids, and aliphatic components, followed by cyclic and alcohol components (Table S1). Contrary to CPMAS NMR results, significantly lower quantities of carbohydrate derivatives were identified among the compounds that were released by pyrolysis. This finding of low pyrolysis yields of carbohydrate derivatives in case of soil humic materials has been linked to the lower efficiency of the thermochemolysis technique to fragmenting polysaccharides (Aquino et al., 2019; Spaccini et al., 2012; Spaccini and Piccolo, 2009). A noticeable release of N derivatives was detected in thermochemolysis of HA samples and was significantly higher in the HA originating from soils that were treated with the RCW than from the controls (Table 5), which is consistent with ^{13}C NPMAS NMR results.

Table 4
Relative contributions (%) of main C structures over chemical shift regions (ppm) and structural indices that were calculated from ^{13}C CPMAS NMR spectra of HA samples.

Samples	Carboxyl-C	Phenol-C	Aryl-C	O-Alkyl-C	C-O/C-N	Alkyl-C	LigR[a]	ARM[b]	HB/HI[c]	A/OA[d]
	190–160	160–145	145–110	110–60	60–45	45–0				
Kika soil										
RCW	11.08	3.17	26.61	20.78	11.39	26.97	3.59	0.62	1.31	1.30
RCW_LV	8.08	2.69	26.39	24.54	14.08	24.22	5.23	0.60	1.14	0.99
PM	7.42	1.22	18.56	34.74	7.43	30.63	6.09	0.30	1.02	0.88
Control	5.90	0.91	14.77	37.05	9.30	32.07	10.23	0.23	0.91	0.87
Initial	11.64	1.34	14.37	34.95	7.11	30.59	5.32	0.24	0.86	0.88
Sékou soil										
RCW	10.75	4.22	23.45	26.57	9.98	25.03	2.36	0.54	1.11	0.94
RCW_PM	14.77	5.51	22.51	25.35	7.29	24.57	1.32	0.56	1.11	0.97
PM	11.74	1.27	22.55	29.49	7.09	27.86	5.60	0.42	1.07	0.94
Control	12.91	1.15	22.55	22.76	8.34	32.27	7.23	0.43	1.27	1.42
Initial	10.24	0.83	19.97	30.89	9.98	28.09	12.10	0.35	0.96	0.91

Only organic treatments effects were presented, as the irrigation volume did not show any effect on the measured parameters (data not shown).

[a] LigR = Lignin ratio (60–45)/(160–145)

[b] ARM= aromaticity index [(160–110)/Σ (45–0) + (110–60)]

[c] HB/HI = hydrophobicity index = [Σ (45–0) + (160–110)/ Σ (60–45) + (110–60) + (190–160)]

[d] A/OA= alkyl ratio (45–0)/(110–60)

Table 5
Relative yield (%) of main thermochemolysis products that are released by humic acids that were extracted from Sékou and Kika soils.

Humic acid	CH	FA	N	G	H	S	Lignin
Kika soil							
Initial	2.2 ± 0.6 a	70.7 ± 2 a	5 ± 0.3 b	12.9 ± 0.1 b	3.3 ± 0.4 d	4.6 ± 1.5c	20.8 ± 1.1 e
RCW_PM	2.1 ± 0.6 a	30.6 ± 0.7c	4.9 ± 0.1 b	15.1 ± 0.3 a	14.5 ± 0.6 b	32.5 ± 0.6 b	62.0 ± 0.3 a
RCW	1.9 ± 0.0 a	30.4 ± 1.1c	9.8 ± 1 a	13.5 ± 2.3 ab	0.2 ± 0.2 e	46.5 ± 0.6 a	60.1 ± 1.9 a
PM	2.1 ± 0.4 a	39.3 ± 0.9 b	0.2 ± 0c	4.2 ± 0.6c	7.5 ± 0.5c	43.6 ± 1.1 a	55.3 ± 1.1 b
Control	2.3 ± 0.6 a	70.1 ± 0.2 a	0.1 ± 0c	0.2 ± 0.0 d	21.2 ± 1.0 a	5.3 ± 0.1c	26.6 ± 0.8c
Sékou soil							
Initial	2.9 ± 0.7 a	47.8 ± 0.4 b	3.7 ± 0.5c	14.7 ± 0.5 a	11.1 ± 0.2 a	20 ± 0.2c	45.7 ± 0.8c
RCW_PM	2.0 ± 0.2 b	41.6 ± 0.8c	4.7 ± 0.2 b	5 ± 0.2c	2.8 ± 0.3c	43.9 ± 0.7 a	51.6 ± 1.1 a
RCW	2.0 ± 0.5 ab	37.2 ± 0.3 d	7.1 ± 0.2 a	13 ± 0.5 b	11.2 ± 0.7 a	27.8 ± 0.6 b	51.9 ± 0.8 a
PM	2.1 ± 0.1 b	49 ± 0.4 b	2.9 ± 0.6 d	1.6 ± 0.5 e	3.2 ± 0.1c	43.3 ± 1.5 a	48.0 ± 1.1 b
Control	2.4 ± 0.2 a	71.8 ± 0.4 a	3.8 ± 0.4 cd	7.1 ± 0.7 d	6.2 ± 0.3 a	10.3 ± 0.6 d	23.5 ± 0.4 d

Only organic treatments effects were presented, as the irrigation volume did not show any effect on the measured parameters (data not shown).
CH, carbohydrate derivatives; FA, fatty acid compounds; N, protein derivatives; G, guaiacyl (lignin derivatives); H, p-hydroxyphenyl (lignin derivatives); S, Syringyl (lignin derivatives); Lignin = G+H+S; RCW, ramial chipped wood; PM, poultry manure; RCW_PM, RCW + PM mixture; Initial, initial condition of the soil; Control, soil without organic amendments. NOTE: means with the same letters within each column indicate no significant difference at $P = 0.05$, according to post-hoc Tukey HSD tests.

The substantial quantity of N-containing products that were released upon thermochemolysis of HA from RCW-based materials may be linked to the progressive insertion of peptide fractions into the humic compounds during the humification stage (Spaccini et al., 2002). As revealed by CPMAS NMR, the broad range of lignin derivatives that were identified among thermochemolysis products of HA was greater for HA from RCW-based materials (RCW and RCW_PM) than from control soils (Table 5). The relative amount and distribution of lignin units that were identified as H for methylated p-hydroxyphenyl, S for syringyl (3, 5-dimethoxy, 4-hydroxyphenyl) and G for guaiacyl (3-methoxy, 4-hydroxyphenyl) units (Kuroda et al., 2002) that were released from the HA samples confirm the source of products detected from RCW of *G. arborea* and *S. latifolius*. As demonstrated in our previous study (Daassi et al., 2020), RCW lignins from *G. arborea* and *S. latifolius* are G-S (guaiacyl-syringyl), which accords with results from HA pyrolysis, confirming incorporation of lignin fragments to a certain extent into HAs. Detection of characteristic lignin-derived compounds in HAs extracted from soils treated with the studied organic amendments could be related to the occurrence of lignocellulosic materials decay induced by their microbial processing in soil and their incorporation in humic substances. The efficient release from the thermochemolysis of cis- or trans-isomers of 1-(3,4,5-trimethoxyphenyl)− 1(3)-methoxy-propene (S17) and 1-(3,4-dimethoxyphenyl)− 1(3)-methoxy-propene (G17) could be associated with the incorporation of slightly decayed RCW into soil organic matter. Conversely, the derivatives from microbially processed RCW included oxidized forms of di- and trimethoxy phenyl products, with benzoic acid (G13, S12), ketonic (G12, S11), and aldehydic (G8, S8) molecules as main components, as has been described by Aquino and coworkers in vermicompost substrates (Aquino et al., 2019). Moreover, the substantial concentration of 1-(3,4,5-trimethoxyphenyl)− 1,2,3-trimethoxypropane (S18) and enantiomers of 1-(3,4-di-methoxyphenyl)− 1,2,3-trimethoxypropane (G18 and G19) confirmed the contribution of lignified tissues that were not fully decayed (as was the case with RCW) to HA formation (Kuroda et al., 2002). Likewise, release of lignin monomers, such as 3-(3,4, 5-trimethoxyphenyl)− 2-propenoic (S9, S10) acid forms and 3-(3, 4-dimethoxyphenyl)− 3-propenoic (G20), could be related mainly to the oxidation of side-chain of syringyl and guaiacyl units. The latter derivatives were higher in HA from the RCW treatment than from the control. In addition, HA in the PM treatment had more lignin than the control HA because the PM contained lignocellulosic residues (sawdust, wood chips mixed with PM) and a significant carbon content (322.1 ± 9.1 $g.kg^{-1}$), which may explain the presence of lignin in the PM. Subsequent decomposition of the PM in the soil could thus explain the high lignin content of the PM treatment compared to the control treatment. The extent of decomposition of RCW 24 months after its residence in soil, was such that the lignin from RCW contributed at 51.9 ± 0.8% and 60.1 ± 1.9% to HA formation at Sekou and Kika sites, respectively (Table 5). Same trends were observed for the RCW_PM and PM. All of these findings suggest important contributions of RCW lignins to the formation of HAs in soil.

The main aliphatic products that were detected in HA from all amended soils were methyl esters of long-chain fatty acids (FA), which were mostly represented by saturated and unsaturated hexadecanoic and octadecanoic acids (Table S1). Our results indicate that the relative aliphatic content among pyrolysis products was significantly lower from HA extracted from RCW-treated soil than was the case for control samples (Table 5). These results are consistent with findings of ^{13}C CPMAS NMR. The predominance of FA with $C > 9$ in all extracted HAs could reveal that they derive mainly from aliphatic biopolymers in wood tissues (RCW and PM), such as cutin and cutan as well as suberin and suberan (Aquino et al., 2019; Chefetz et al., 2002; Kuroda et al., 2002; Spaccini et al., 2019). The derivatives of these aliphatic biopolymers, some of which are quite important in RCW that contains high proportions of bark, are related to the stabilization of soil humic acids due to their ability to structure ester-bridge bonds, thereby enhancing intermolecular hydrophobic interactions (Monda et al., 2017).

3.3. *Effect of organic amendment in soil fungal community*

3.3.1. *Fungal community taxonomic classification and diversity*

In total, > 4.5 million high-quality fungal sequences were filtered and assigned into sequence identity-based clustering (thereby producing Operational Taxonomic Units, OTUs). OTU diversity was assessed by rarefying the sequences to a sufficiently even depth of 10000 (1687 total OTUs). At the Sékou site, the number of shared ("core") OTUs (88 OTUs, 6.1%) among all amended soils was greater than those that were shared in amended soils from the Kika site (68 OTUs, 4.0%) (Fig. 5).

OTU numbers under initial soil conditions were greater than those of amended (RCW, RCW_PM and PM) and control soils at Sékou while those in the initial soil conditions at Kika were higher than those in the RCW_PM, PM and control treatments. However, at the Sékou site (Fig. 5 B), the OTU number in RCW_PM soil (93 OTUs, 6.42%) is greater than those in RCW (84 OTUs, 5.8%) and PM (77 OTUs, 5.3%) soils. Similarly, at the Kika site (Fig. 5 A), RCW (131 OTUs, 7.7%) had an OTU number that was greater than those in PM (121 OTUs, 7,17%) and RCW_PM (97 OTUs, 5.8%) soils. The relative distribution of OTUs in amended soils on each site could be attributed to the intrinsic characteristics of the types of organic amendments (RCW vs. PM), which are differentiated by their chemical and structural composition, together with initial soil conditions.

Relative fungal abundance, at both phylum and genus levels, from

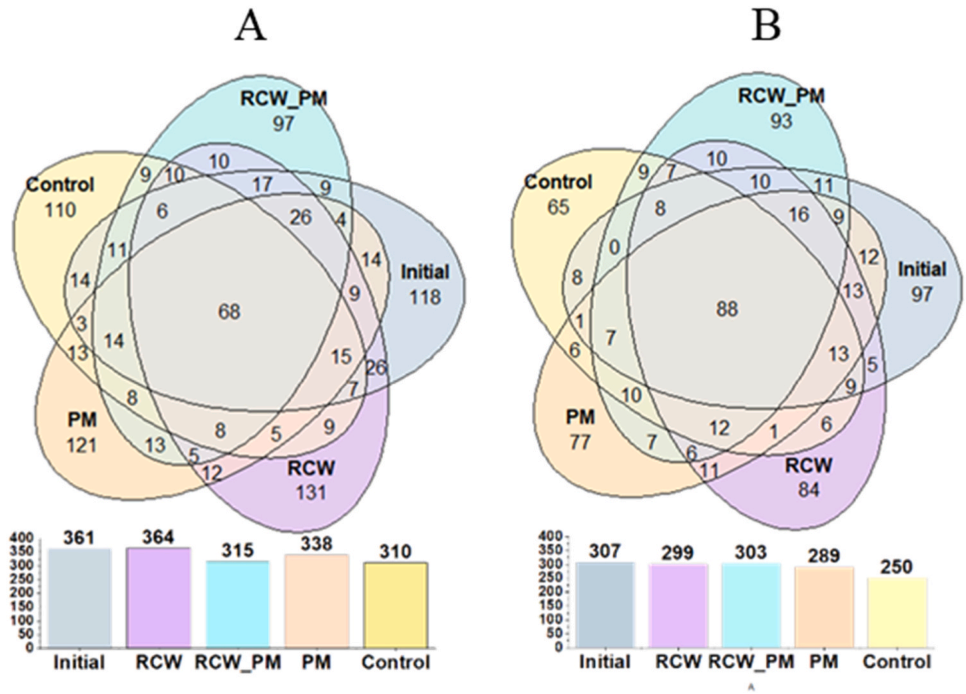

Fig. 5. Shared fungal OTUs among amended soils using Venn diagrams. A: Kika site, B: Sékou site. Initial, initial condition of soil; Control, soil without amendment; RCW, ramial chipped wood; PM, poultry manure; RCW_PM, RCW + PM mixture.

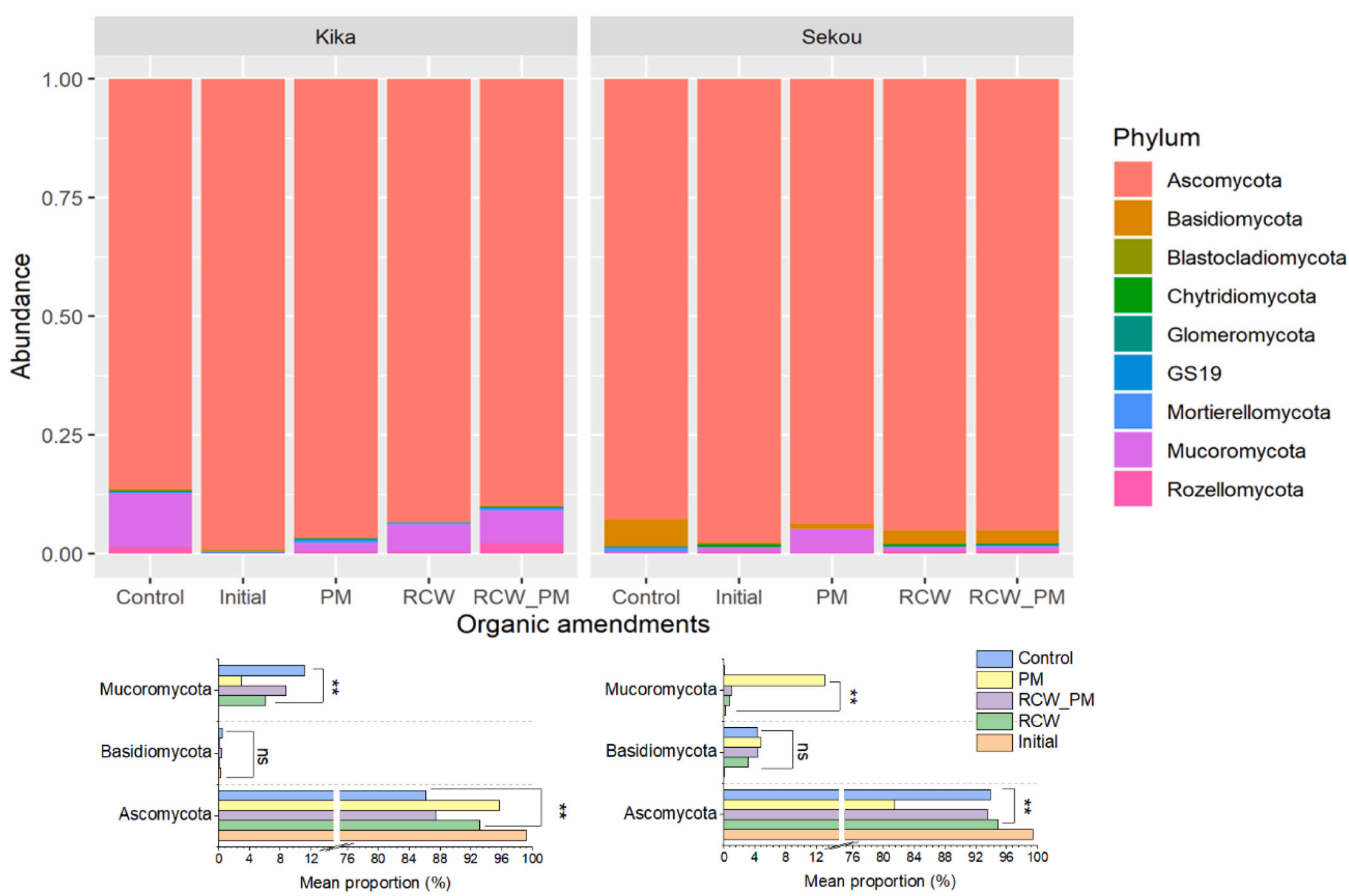

Fig. 6. Relative abundance of fungal phyla in amended soil and mean proportions of the three most abundant phyla. Initial, initial condition of soil; Control, soil without amendment, RCW: ramial chipped wood; PM, poultry manure; RCW_PM, RCW + PM mixture. ns: non-significant, * *: significant, significant level $P < 0.05$ with Tukey HSD tests.

amended soils at the Sékou and Kika sites is illustrated in Fig. 6, S3 and S4. In all amended soils, nine major phyla were identified: Ascomycota (92.71%); Mucoromycota (4.23%); Basidiomycota (1.97%); Rozellomycota (0.53%); Mortierellomycota (0.42%); Chytridiomycota (0.09%); GS19 (0.03%), Glomeromycota (0.02%); and Blastocladiomycota (0.01%). The top three phyla accounted for over 98.5% of total sequences at both Sékou and Kika sites. This result is corroborated by published studies revealing that the Ascomycota is the most dominant phylum in shrublands and grasslands (Bello et al., 2021; Challacombe et al., 2019; Ezeokoli et al., 2020; Wang et al., 2020). Previous research has also reported the phyla Basidiomycota, Mucoromycota, Mortierellomycota, Rozellomycota, and Chytridiomycota in soils (Bello et al., 2021; Ezeokoli et al., 2020; Wang et al., 2020), which were also identified in this study. Ascomycota and Mucoromycota at Kika with RCW and PM treatments produced the highest relative abundances for these phyla, while at Sékou for these same phyla, RCW, RCW_PM and control soils produced a greater relative abundance than that for PM (Fig. 6). No noticeable difference was observed in Basidiomycota. Ascomycota are reported to play an important role in carbon and nitrogen cycles in tropical ecosystems (Wang et al., 2022, 2020). Their substantial abundance in RCW-amended soil could contribute to RCW biomass decomposition, lignin persistence, soil stability against erosion, and direct interactions with plants, by acting as pathogens and endophytes that disrupt and disassemble plant tissues (Challacombe et al., 2019; Ezeokoli et al., 2020; Yu et al., 2011).

At the genus level, patterns that were similar to the phyla were found within the fungal community. The top four genera in amended soils were: *Penicillium, Talaromyces, Lectera* and *Aspergillus.* These accounted for more than 97.5% of total fungal sequences, all of which belong to the phylum Ascomycota (Fig. S5). At the Kika site, RCW and PM produced the greatest relative abundance of *Penicillium, Lectera* and *Aspergillus*, while the highest relative abundance of *Talaromyces* was found with RCW_PM (Fig. S5). At the Sékou site, the highest abundances of *Penicillium, Talaromyces* and *Lectera* were reported for the controls, RCW and PM, respectively. No significant response was detected in the genus *Aspergillus* to the applied treatments. Significant differences that were observed among the top four Ascomycota genera in amended soil relative to that of the control showed that fungal communities were inversely affected by the source of the amendment, with a high abundance due to RCW. Studies have indicated that soil organic amendment exerts a significant influence on soil microbial community composition and dynamics (Bello et al., 2021; Challacombe et al., 2019; Dhaliwal et al., 2019; Lazcano et al., 2013). The genera *Penicillium* and *Talaromyces* are ubiquitous in soil and are important drivers of phosphorus cycling (Hao et al., 2020; Raymond et al., 2019). Likewise, *Talaromyces* has been reported to be a cellulose-degrading fungus producing soluble pigments and cellulase for the degradation of ligno-cellulosic biomass in soils (Datta et al., 2017; Zhang et al., 2020). The greater relative abundance of *Penicillium* and *Talaromyces* in RCW-amended soil could account for increased assimilable phosphorus content in the RCW-based treatments (Fig. 2 and Fig. 3). The relative abundance of Basidiomycota that were obtained in Sékou soil could be attributed to both direct and indirect effects of RCW (improved soil chemical properties, water retention, nest of many fungi against predators) (Barthès et al., 2010; Robert et al., 2014; Valet and Ozier-Lafontaine, 2016). However, the low relative abundance of basidiomycetes recorded for soils under RCW treatments compared to other treatments showed that these fungi have not yet contributed significantly to the degradation of RCW since white-rotting Basidiomycota are known to efficiently degrade lignocellulosic materials (Datta et al., 2017; Gul et al., 2015; Thevenot et al., 2010). Therefore, lignin depolymerization would be low at this stage since lignin concentration in RCW increased during RCW decay within the timeframe of this study as shown by Daassi et al. (2021).

3.3.2. *Influence of organic amendments and environmental factors on fungal alpha diversity indices*

The Shannon index of diversity, Pielou's evenness, OTU richness, and the Faith phylogenetic diversity index for amended soil fungal OTUs did not significantly differ between treatments at both Sékou and Kika soils (Table 6).

Yet, canonical correspondence analysis (CCA) illustrated relationships between the fungal community and soil chemical variables, given that the latter are reported to be important drivers of soil microbial structure (Bello et al., 2021; Ezeokoli et al., 2020; Gul et al., 2015). At Kika, there is no distinct variation among treatments, other than RCW, for the fungal community based on CCA axis 1 (Fig. 7 A). This result revealed the occurrence of variation in the fungal community of the two study sites, which was prone to being incurred by soil chemical properties (Fig. 2 and Fig. 3), the contrasting initial chemical compositions of the RCW from *G. arborea* and *S. latifolius*, and the differing climate conditions of the two sites.

This finding corroborated results of previous work by Yu et al. (2011), Gul et al. (2015) and Bello et al. (2021), who reported that climate conditions and seasonal application of organic amendments contributed to changes in the soil microbial community of tropical soils. Our work focused upon the analysis of the soil properties among treatments before and after two vegetables growing seasons in the same rainy period. Yet, some studies have reported that changes in soil microbial diversity are often between two different seasons than between two different treatments (Anis and Arya, 2021; Luo et al., 2019). In tropical soils, seasonal variation in microbial diversity and biomass carbon was observed to be minimal during the rainy season and maximal during the dry season (Anis greater and Arya, 2021; Luo et al., 2019), respectively. As summarized in Fig. 2 and Fig. 3, RCW amendment provided more soil organic carbon at the Kika site than at the Sékou site, which may be responsible for the reduced effect of the organic amendment on fungal diversity. Based on CCA, the biplot model was significant ($P = 0.007$) for the fungal community in Sékou soil (Fig. 7 B), but slightly significant ($P = 0.051$) in Kika soil (Fig. 7 A). More precisely, 58% and 44% of variation in fungal community distributions of Kika and Sekou soils were respectively explained by environmental variables. Environmental variables that were fitted in the step-wise CCA model at the Sékou site showed that bulk density (BD) ($r^2 = 0.75$, $P = 0.001$), CEC ($r^2 = 0.582$, $P = 0.001$), E4/E6 ratio ($r^2 = 0.488$, $P = 0.001$), SOC ($r^2 = 0.359$, $P = 0.001$), K ($r^2 = 0.313$, $P = 0.002$), C/N ratio ($r^2 = 0.243$, $P = 0.010$), and phosphorus (P) ($r^2 = 0.151$, $P = 0.045$) significantly

Table 6
Mean alpha diversity indices for fungal communities across amended soils in the Kika and Sekou sites.

	Kika				Sekou			
Treatments	Shannon Kika soil	Pielou	OTU richness	Faith PD	Shannon Sékou soil	Pielou	OTU richness	Faith PD
Initial	4.5 ± 0.3a	0.7 ± 0a	78 ± 17.2a	5.7 ± 1.5a	3.7 ± 0.4a	0.6 ± 0a	68.8 ± 13.2a	6.9 ± 1.3a
RCW	3.9 ± 0.3a	0.6 ± 0a	70.6 ± 9.4a	7.6 ± 1a	4.1 ± 0.2a	0.7 ± 0a	68.6 ± 11.1a	4.9 ± 0.7a
RCW_PM	4.2 ± 0.3a	0.7 ± 0a	70.7 ± 10.2a	7.7 ± 1a	4.4 ± 0.2a	0.7 ± 0a	83.8 ± 11.5a	6.1 ± 0.7a
PM	3.7 ± 0.3a	0.6 ± 0a	66.6 ± 9.4a	7.7 ± 1a	4.5 ± 0.2a	0.7 ± 0a	78.3 ± 11.5a	5.7 ± 0.7a
Control	4.1 ± 0.3a	0.7 ± 0a	75.9 ± 10.2a	8.9 ± 1a	4.4 ± 0.2a	0.7 ± 0a	72.5 ± 11.1a	6 ± 0.7a

Values (means ± SEs) within a column with the same letters do not significantly different ($P < 0.05$) based on post-hoc Tukey HSD tests.

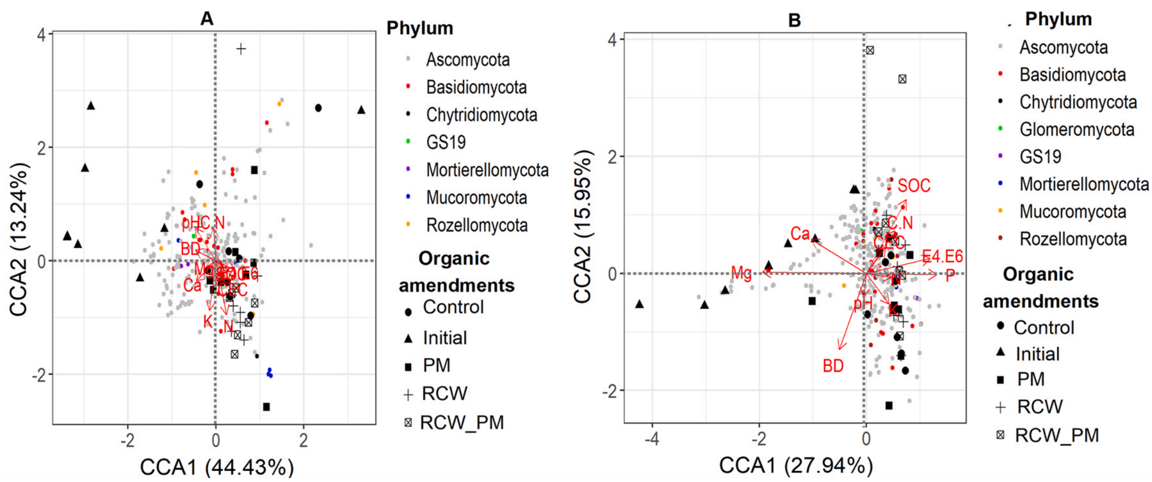

Fig. 7. Canonical correspondence analysis (CCA) showing the relationship between soil chemical properties and fungal community in Kika soil (A) and Sékou soil (B). CCA model for soil is significant ("anova.cca ()", $P < 0.05$).

affected the fungal community. At Kika, Mg ($r^2 = 0.570$, $P = 0.001$), SOC ($r^2 = 0.363$, $P = 0.001$), BD ($r^2 = 0.329$, $P = 0.001$), P ($r^2 = 0.248$, $P = 0.008$), E4/E6 ratio ($r^2 = 0.233$, $P = 0.010$), and Ca ($r^2 = 0.199$, $P = 0.021$) influenced significantly fungal community. These results are in agreement to those found in published research reporting that BD, SOC, phosphorus (P), C/N ratio, CEC, Ca, Mg and the E4/E6 ratio of humic acid exhibited significant correlations with fungal abundance and indices in soils (Bello et al., 2021; Chen et al., 2018; Ezeokoli et al., 2020; Wang et al., 2020; Yu et al., 2011). These results could be related to the application of organic amendments based on ramial chipped wood, which increased SOC (Fig. 2 and Fig. 3), thereby improving soil macro- and micro-nutrient availability. The latter nutrients act as co-factors for several enzymatic systems that metabolize various organic molecules, including carbohydrates, lignins, lipids, proteins and nucleic acids (Bello et al., 2021; Dhaliwal et al., 2019).

Correlations between soil chemical properties and alpha-diversity indices of fungal communities showed at Sékou site that the Shannon index was negatively correlated with total nitrogen (Spearman rank correlation: $r_s = -0.38$, $P = 0.02$), while Pielou evenness was negatively correlated with nitrogen ($r_s = -0.41$, $P = 0.01$), but positively with C/N ratio ($r_s = 0.45$, $P = 0.004$). Likewise, Faith PD was negatively correlated with potassium ($r_s = -0.41$, $P = 0.01$) and magnesium ($r_s = -0.35$, $P = 0.03$). No significant correlations were obtained between soil parameters and alpha-diversity indices at the Kika site. Pearson heatmap correlation analysis at both Sékou and Kika sites between physiochemical soil variables and major fungal phyla are shown in Fig. S9 and S10. The results show that at Sékou site, Ascomycota and Basidiomycota had significant negative correlations with soil bulk density and organic carbon, while Mortierellomycota was positively correlated with Ca, Mg, P and SOC. Mucoromycota was also positively correlated with K, whereas pH was positively correlated with GS19 and Basidiomycota. At the Kika site, Ascomycota and Rozellomycota had positive correlations with pH, while Chytridiomycota correlated positively with Ca, Mg N and SOC. The various significant effects of edaphic factors on phyla that were identified in this study could explain the distribution of fungi on soils according to the type of organic amendment that was used (RCW and PM). These phyla have been reported in several studies as the major drivers of element transformations making nutrients available from soil organic amendments and controlling nutrient turnover in most soils (Bello et al., 2021; Lazcano et al., 2013; Wang et al., 2020).

4. Conclusion

Application of organic amendments depending on their type and composition had a variable effect on soil chemical and fungal characteristics in Benin sandy loam soils. Our study confirmed that RCW application improves soil chemical properties and contributed by more than 56% to SOC storage, ranging from 12.68 ± 3.18–41.7 6 ± 3.18 Mg/ha at the Sékou site and from 22.37 ± 3.65–45.83 ± 3.65 Mg/ha at the Kika site, after 24 months of in situ trials. Irrigation volume had a variable effect on soil chemical properties and a regulated RCW amendment effect on SOC and P contents at Sékou as well as on Ca content at Kika site. In soils amended with RCW-based treatments, lignin-derived compounds strongly contributed to humic acid formation as documented by humic acids yields and their higher contents of aromatic structures. In addition, the application of RCW and PM did not affect fungal diversity and richness, but stimulated certain soils fungal taxa, especially the Ascomycota, Mucoromycota and Basidiomycota. At the level of genus, Penicillium, Talaromyces, Lectera and Aspergillus were the most abundant taxa on amended soils, establishing associations that contributed to soil nutrient cycling. Based on carbon accumulation, nutrient availability, HA yield and structure, and fungal community abundance, RCW, in combination with other organic amendment or as sole amendments are recommended for improving the health of sandy loam soils in Benin and elsewhere in sub–Saharan Africa.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.still.2023.105974.

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