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Impact of Biochar Applications on Tropical Soils under Different Land-use Regimes

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Abstract

The application of biochar to agricultural soils can either be beneficial or detrimental, as well as no clear effect to soils and crops. Therefore, the aim of our study was to investigate the effects of biochar addition on soil chemical and biological properties and nutrient leaching in three tropical soils with different types of land-use (forest, non-intensive and intensive farming). The soils were amended with and without 2% coconut shell (CS) and rice husk (RH) biochars by weight and incubated for up to 360 days. To assess the impact of biochar on soil leaching, 27 unplanted soil columns from the same types of land-use were also amended with and without 2% CS and RH biochars by weight. Five leaching experiments were conducted by passing through 100 ml of deionised water via each of the glass columns containing soil. The biochar addition significantly increased ($P<0.05$) the soil pH and total carbon, but had a marginal effect on CEC and had a limited effect on microbial activity. Biochar treatments reduced ammonium leaching in the forest soil, but had no clear effect on the other two soils. Our data showed that biochar application at a lower rate can ameliorate soil acidic conditions, enhance carbon sequestration and adsorb ammonium ion. However, the success depends on soil and biochar properties and land-use. The biochar samples studied have a limited capacity to reduce nitrate and phosphate leaching due to high biochar phosphate content.

Keywords: nutrients, leaching, carbon-14, mineralization, ammonium, phosphate, nitrate

1. Introduction

Soils of the tropics are dominated by Oxisols and Ultisols (Ishak and Jusop 2010), which are highly-weathered soils with low pH and a mineralogy dominated by quartz, kaolinite clays and higher oxides of iron and aluminum (Zhao et al. 2014). Tropical soils often have a low cation exchange capacity (CEC) and, in some cases, higher anion exchange capacity (AEC), (Yamato et al., 2006; Masulili et al. 2010). A higher AEC may result in the loss of ammonium ions through leaching and runoff. Hence, nitrogen fertilizer applications have to be much higher. The efficiency of the fertilizer utilization is lower, due to the leaching process (Ludwig et al. 2001). Furthermore, long-term intensive farming of arable land has decreased the carbon content of the soils of the tropics (Lal 2009), adversely affecting their fertility. According to Nottingham et al. (2020), soils in the tropics have a high tendency to lose more carbon as CO_2 following warming climates.

Increasing the content of soil carbon is important to improve the stability of soil structure, enhance water-holding capacity and facilitate water movement in the soil. It also helps to increase carbon sequestration and reduce soil loss (Victoria et al. 2012). Ultisols and Oxisols are known to have a low percentage of organic carbon (1% to 2%). The low level of organic carbon can be insufficient for good crop performance (Ishak and Jusop 2010). In addition, low level soil organic carbon limits soil aggregation and carbon sequestration which undermines sustainable soil health (Singh et al. 2016). Consequently, organic amendments can be applied to restore appropriate soil organic carbon levels in order to promote sustainable soil quality and crop productivity. Manure and compost are sources of organic carbon easily available to farmers. However, these amendments may not have optimal performance influence in soil recalcitrant carbon pools (Yanardağ et al. 2015). Biochar is an

organic amendment that has been shown to improve soil quality by enhancing the recalcitrant carbon pool and resisting carbon degradation by microbes (Agegnehu et al., 2017; Mensah and Frimpong 2018). Biochar can be produced from various organic resources, including unwanted agricultural by-products, animal dung and wood. These materials are usually burned in a secure chamber, creating a condition with restricted or zero oxygen (Lehmann and Joseph 2009). The application of biochar to soil has been practiced in the past. The fertile soils found in the Amazon, called Terra Preta or dark earth, were generated by aboriginal societies through the use of biochars (Glaser and Woods 2004). This type of soils (dark earths) have also been found in Africa (Fraser *et al.*, 2014). Furthermore, research in biochar studies discovered that biochar has many advantages as a soil conditioner. For instance, biochar can increase pH and eventually be able to increase the CEC of the soil (Masulili et al. 2010; Sukartono et al. 2011).

Amending soil with biochar increases crop productivity and soil quality in subtropical regions where soil pH is normally very low and soil texture is coarse (Farhangi-Abriz et al., 2021). Lin *et al.* (2015) found that, adding biochar to soil at the rate of 16t ha^{-1} improved the development of wheat plants by 27.7%. Also, another study on the application of high rates of biochar (50 t ha -1) with nitrogen fertilizer to clay loam (low organic matter – 0.84%) soils was conducted by Jalal et al. (2020). The study was carried out over two years and involved maize and legume production. Results showed that wood biochar enhanced the yield of maize, biomass production and nitrogen use efficiency. However, some papers in the scientific literature have questioned the efficacy of biochar. Jay et al. (2015) showed that, amending soil with biochar improved crop yield and decreased nutrient leaching in studies conducted in temperate areas. These results are, however, not always positive (Van Zwieten et al. 2010). Other long-term biochar studies revealed that nitrate, ammonium, dissolved organic C and nitrogen did not affect N mineralization. Also biochar application caused little and temporary changes to the agricultural ecosystem (Jones et al. 2012; Quilliam et al. 2012). Crop performance for three growing cycles also showed a marginal effect after biochar addition to the soil (Jones et al. 2012). The drawback of biochar application is the presence of phytotoxic substances. These substances, such as phenolic compounds, formic acids or acetic acids and polycyclic aromatic hydrocarbons (PAHs) have been discovered from newly produced biochar (Quilliam et al. 2013b). If present in substantial amounts, these substances may affect plant performance (Busch et al. 2012), as well as become toxic to soils, plants and microbiomes (Quilliam et al. 2013b). In addition, Bruun et al. (2008) and Zhang et al. (2014) claimed that, there was no effect on the microbial biomass and activity, when biochar was added to soil, but Dempster et al. (2010) discovered that amending soil with biochar resulted in a reduction in microbial biomass. Indeed, differences in the feedstocks of biochar and in the characteristics of the soil influence the eventual result of biochar application.

Most biochar studies in the past focused on soils in temperate regions (Jones et al. 2012; Quilliam et al. 2013a). Some research work, however, examined the effects of biochar on tropical soils (Alling et al. 2014) or the long-term outcomes of biochar application (Jones et al. 2012; Quilliam et al. 2012). Therefore, the purpose of this study was to explore an aging effect of soil and biochar mixtures on soil properties in tropical region. Our hypotheses were that amending tropical soils with biochar would decrease nutrient leaching and enhance the biological and chemical characteristics of the soils.

2. Materials and Methods

2.1 Chemicals and Soils

^{14}C glucose was purchased from Sigma Aldrich Co. Ltd. UK. Goldstar multipurpose liquid scintillation cocktail was procured from Meridian, UK. Sample oxidizer cocktails (Carbotrap and Carbocount) were obtained from Meridian UK, and combustaid was obtained from Perkin Elmer, USA. Ammonium acetate, sodium acetate, chloroform ($CHCl_3$), potassium sulphate and sodium hydroxide were supplied by Fisher Scientific, UK.

Three tropical soils (Spodosols) were used in this study. A secondary forest soil (sandy clay loam), a non-intensively-farmed soil (sandy clay) and an intensively-farmed soil (sandy clay) were chosen based on differences in types of soil degradation (Table 1 and Supplementary Figure 1). An accumulation of Al and Fe in the subsoils is usually found in Spodosols which are acidic (pH < 5.5). In the secondary forest soil used in this study which is a sandy clay loam, logging activity has happened for several years. Meanwhile, soil that has been cleared for agricultural purposes only once is the non-intensively-farmed soil. The intensively-farmed soil which was the soil that has been planted with different crops under a rain shelter for a few years was also used in this study. All of the soils were sampled from the Cameron Highlands District, Pahang in central Peninsular Malaysia (Supplementary Figure 1). At an altitude of 1070 – 1830 m above sea level, the mountainous area has slopes between 10 and 35 ^{o} (Abdullah et al., 2001). Soil was obtained from 5 random samples with an auger to a depth of 10 – 15 cm within an area of 27.5 m^2. The soil samples were then composited and kept field-moist in a cold

room at approximately 9 ^{O}C before being shipped to the United Kingdom for chemical and biological soil analyses. The soil samples from Cameron Highlands were then sieved through a 5 mm mesh to remove plant residues and stones prior to the experiment.

2.2 Biochars

Coconut shell (CS) and rice husk (RH) biochars, were used in this study. The RH biochar was obtained from Tanjung Karang, Selangor Malaysia. It was produced by a rotary husk furnace which resulted in a higher biochar yield at 900^{O}C to 1000^{O}C within a short period of time. On the other hand, the method which produced CS biochar is called slow pyrolysis, where the CS was heated in a drum. This burning process was conducted approximately 6 to 8 hours and the temperature adjacent to the drum can reach up to 400^{O}C. RH and CS biochar samples were crushed and sieved using a 2 mm mesh in the laboratory. Then, the soil samples were mixed with 2% of the RH and CS biochar samples by weight. Soil not mixed with biochar act as a control treatment. All samples were then incubated at the-five time point (0, 60, 120, 240 and 360 days) with 45% of moisture content and at a constant temperature of approximately 21^{O}C. This created an environment that closely resembled the conditions (temperature and moisture content) from which the soils were collected. The soil samples were then kept in screw-capped glass jars. Following methods by Kandeler (2007), the soils were dried in the fume hood and sieved using a 2 mm mesh to obtain suitable soil aggregates for the soil chemical and biological analyses at each time point.

2.3 Nutrient Leaching Study

Soil samples were placed into 27 glass soil columns. Each column has a diameter of 5 cm and is 20 cm long. Each column of soil was adjusted to 1.2 g cm^{-3} bulk density. At the bottom of the funnel used, a glass wool layer was inserted to prevent soil particulate blockage. On the soil surface, another layer of glass wool was inserted to protect the surface structure during the leaching process. Five leaching events (0, 60, 120, 240 and 360 days) were conducted by leaching 100 ml of deionized water through each of the soil columns. Erlenmeyer flasks were used to collect the leachate and then stored at 4 ^{0}C for two to three days before analysis. The leachates were then determined for ammonia, nitrate and phosphate contents using a Bran + Luebbe Autoanalyzer 3.

2.4 Soil Biological and Chemical Analyses

For biological experiments, the methodology used was substrate-induced respiration (SIR) as described by Hamer and Marschner, (2002) and; Kandeler, (2007). On days 0, 60 and 120 incubation time, samples containing 20 g wet weight of soil were mixed with 3mM of glucose solution with a radioactivity of 733 Bq. Meanwhile, on days 240 and 360 of incubation time, the soil samples had a radioactivity of 1086 Bq (Doick and Semple 2003). 1 ml NaOH (1 M) solution was added into a 7 ml vial attached to the respiratory bottle's lid to allow it to be suspended and therefore trap $^{14}CO_2$ generated by the $^{14}CO_2$ mineralization process. The samples were mixed and shaken with a shaker at 100 rpm. Then, the vials were replaced every 1, 2, 3, 4, 6, 8, 10 and 12 hours; and another 24, 48, 72, 96 and 120 hours. During the sampling, the vial containing 1 M of NaOH solution was detached from the respiratory bottle and the surface of the vial was cleaned using acetone to discard any residues of $^{14}CO_2$. Then, 5 ml of liquid scintillant cocktail was added into the vial. The mixture was incubated overnight in the cabinet. ^{14}C activity in the vial was then analyzed using a liquid scintillation analyzer (Canberra Packard Tri-Carb 2250A).

For microbial biomass determination, the soil slurry was sampled at the final day sampling of the SIR experiment. The method used to measure microbial biomass is fumigation and non-fumigation methods. Where 20 ml of 0.5 M K_2SO_4 was added to the non-fumigation sample. The samples were mixed and shaken with a shaker for 30 minutes at 100 rpm. Then, 5 ml of filtered supernatant was placed into a 20 ml vial. Before counting using liquid scintillation, 15 ml of liquid scintillant cocktail was also added into the vial and the samples were kept in a cabinet overnight. Subsequently, the other sample was fumigated in a desiccator, circled with a wet tissue. 75 ml ethanol-free chloroform ($CHCl_3$) was inserted at the middle of the desiccator. The desiccator was emptied until the $CHCl_3$ had boiled vigorously for 120 seconds. The residual $CHCl_3$ gas was discarded after 24 hours by repeated five- or six-fold evacuation. Furthermore, the samples were extracted and counted using a liquid scintillation analyzer like aforementioned. Finally, again the remaining soil samples from SIR, non-fumigation and fumigation experiments were used to measure the ^{14}C-glucose activity remaining in the soil. This was assessed through combustion on a sample oxidizer (Packard, Model 307) for 3 minutes.

pH was measured using a pH meter model PHM 220 which was calibrated using buffers at pH 4.0 and 7.0. The cation exchange capacity (CEC) was measured using 1 M ammonium acetate. This reagent (ammonium acetate) adsorbed cations (Na) which were retained by the soil (Hazelton and Murphy 2007). Using flame photometry, the concentration of displaced Na from the soils was obtained. Meanwhile, total carbon and nitrogen were

determined using an elemental analyzer (Elementar Vario EL) via a dry combustion method. Where soil samples were dried in the constant room temperature at 21^{O}C for about two to three days. The soil samples were then ground using a pestle and mortar. The ground samples of approximately 30mg were weighed into tin cups, which were subsequently loaded into an auto-sampler, which dropped the sample into a combustion column maintained at 950^{O}C. The sample and cup were flash combusted in a temporarily enriched atmosphere of oxygen. The combustion products were carried by a carrier gas, which is helium, past an oxidation catalyst of copper oxide kept at 950^{O}C inside the combustion column.

The combustion products such as CO_2, CO, N, NO and water passed through a reduction reactor in which hot metallic copper with the temperature at 550^{O}C that removed excess oxygen and reduced N oxides to N_2. These gases, including with CO_2 and water, were next went through sicapent to remove water then through a chromatographic column to a thermal conductivity detector. The detector generated an electrical signal proportional to the concentration of N or C present. This signal was graphed on a built in recorder and ported to a computer, which integrated the area under each curve and converted it to concentrations after each sample was run. Finally, the results were given in percentages.

2.5 Statistical Analysis

Statistical significance was determined using one-way ANOVA. There were 9 treatments and three replicates, with sum of 27 samples for each time point. Multiple mean comparison was conducted by applying the Holm-Sidek test at $P < 0.05$. We also used a non-parametric statistical test, the Kruskal-Wallis test, for values that were not normally distributed. To determine significant differences between the treatments for non-distributed values, Tukey test was applied. All statistical tests were run using SigmaStat v. 3.5 (Systat Software Inc.).

3. Results and Discussion

3.1 pH and CEC of the Soil

Biochar addition to soil significantly affected the pH of the soil. Amending soils with CS and RH biochar samples increased ($P<0.05$) their pH significantly (Supplementary Figure 2). Even though the pH of the soil increased, the application of biochar to the soils dropped the soil pH over time ($P<0.05$) due to loss of basic anions from the soils during microbial decomposition activities. As presented in Table 1, both biochars had a high pH. The pH of CS is 8.33 and that of RH is 8.46. According to Novak et al. (2009) and Masulili et al. (2010), adding a biochar with high pH is sufficient to raise the value of the soil pH and improve the condition of an acidic soil.

Table 1. Physical and chemical characteristics of soils and biochars

Soils	Secondary forest soil	Intensively farmed soil	Non-intensively farmed soil	CS biochar	RH biochar
Latitude / °	4.4710	4.4673	4.4668	-	-
Longitude / °	101.3907	101.3857	101.3871	-	-
pH	4.62	5.03	5.52	8.33	8.46
CEC/meq 100 g^{-1}	9.7	9.4	5.8	31.17	43.28
Carbon %	3.42	1.64	1.08	72.95	38.64
Nitrogen %	0.18	0.22	0.07	0.53	0.53
C/N Ratio	19.05	7.38	14.99	139.71	72.59
Inorganic P/mg g^{-1}	0.06	1.85	0.2	0.39	1.75
Clay %	28.63	35.06	33.89	-	-
Silt %	11.01	18.09	18.08	-	-
Sand %	60.36	46.86	48.03	-	-
Texture	Sandy Clay Loam	Sandy Clay	Sandy Clay	-	-

Application of biochar in this present study had a marginal effect on the CEC of the soil. For example, RH biochar had a small effect on the CEC in the secondary forest soil and in the non-intensively-farmed soil. Meanwhile, in the intensively-farmed soil, there was no effect on CEC after biochar application. In addition, the CS biochar did not affect the CEC in any of the soils studied (Supplementary Table 1). Amending soil with both biochars also did not significantly increase the CEC ($P>0.05$). However, some other research results showed that biochar addition to soil elevated the value of CEC (Masulili et al. 2010). In another study, Novak et al. (2009) found that the application of 0.5, 1.0 and 2.0% pecan-shell biochar had only a marginal effect on the CEC of

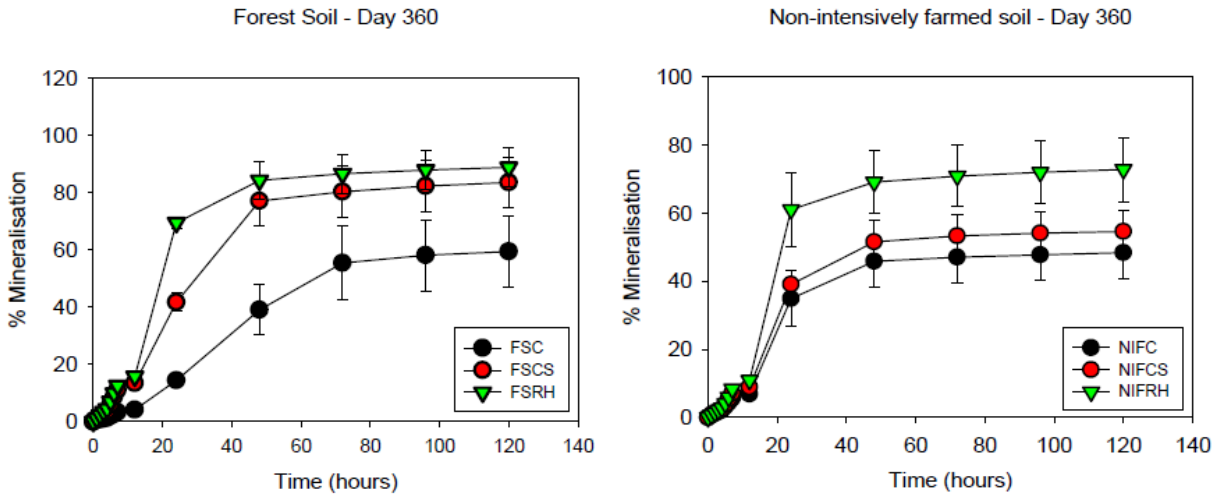
soils. The authors believed that the temperature of biochar pyrolysis influenced the biochar's ability to increase soil CEC. There are often low negative surface charges on high temperature biochar, due to dehydration and de-oxidation of the H- and O-containing functional groups and can have little or no effect on the CEC of soils (Tomczyk et al. 2020). Owing to the high production temperature (RH biochar) and long holding time (CS biochar), our biochar exhibited low CEC values in comparison to literature with limited capacity to exert CEC influence to low CEC soils.

3.2 Carbon, Nitrogen and Phosphate in Soils

Biochar addition to soils increases the carbon content. The highest increase was observed in the CS biochar-amended forest soil (8.2%) (Supplementary Table 2). Nevertheless, the results exhibited a gradual reduction in carbon content over time, owed to the decomposition of the labile carbon pool within the biochar-soil matrix. CS biochar had very high carbon content (72%) that ensured a doubling carbon content effect in all soils over the incubation period when contrasted to un-amended soils. The RH biochar also increased significantly ($P<0.05$) the soil carbon content of all soils, but to a lesser extent than CS biochar due to the lower carbon content. Nevertheless, there was a significant decline in carbon content following 360 days of incubation period in forest (5.98-4.22%), non-intensively (3.13-2.44%) and intensively-farmed (2.67-2.28%) soils amended with RH biochar. In contrast, carbon content in CS biochar-amended soils decreased in forest (8.15-6.35%), remained stable in non-intensively (3.71-3.68%) and increased in intensively-farmed (2.50-3.47%) soils (Supplementary Table 2). This clearly shows that biochar can support carbon sequestration in soils, but the success of application largely depends on soil characteristics, biochar properties and land-use.

3.3 Mineralization of ^{14}C-glucose to $^{14}CO_2$ and Incorporation of ^{14}C-glucose into Microbial Biomass

The extent of ^{14}C-glucose mineralization in the three soils was not continually constant. Generally, the extent of mineralization of ^{14}C-glucose peaked ($P<0.05$) at the last of the incubation period in all the treatments (Figures 1a, b and c; and Tables 2-4). Furthermore, adding both biochar to the soils did not show any major change during the incubation period. It was observed that, after 120 days, the extent of change in ^{14}C-glucose in the forest soil amended with RH biochar (62.22 ± 6.40) was significantly higher ($P<0.05$) than that in the CS-biochar-amended forest soils (45.01 ± 6.05) (Supplementary Figure 3 and Table 2). The maximum rates of ^{14}C-glucose mineralization after 360-day incubation were observed to be higher in the forest soils amended with RH biochar, (4.47% h^{-1} ± 0.23), in comparison with the rates of the forest soil amended with CS biochar (2.35% h^{-1} ± 0.20), as well as the rate with no biochar (1.03% h^{-1} ± 0.30) (Table 2). Overall, the uptake of ^{14}C-glucose into the microbial biomass exhibited no constant trend in all the soils and treatments (Tables 2-4).



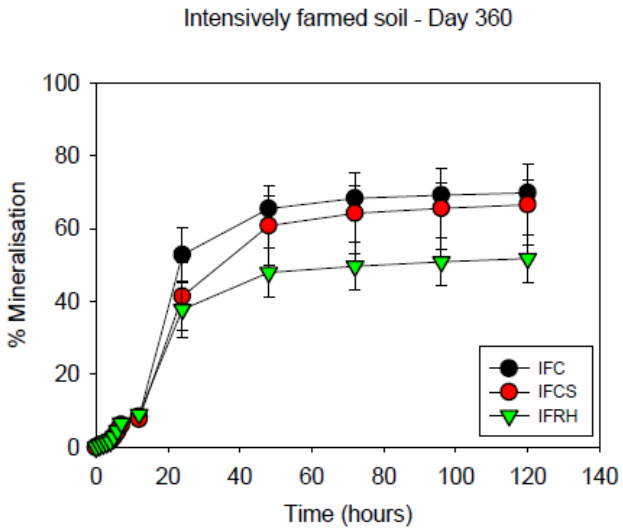


Figure 1. Mineralization of 14C-glucose on day 360 of the a) forest b) non-intensively farmed and c) intensively-farmed soils amended with CS and RH biochar and without biochar. Error bars are SEM (n=3)

Table 2. Maximum rate, ^{14}C extent mineralization, ^{14}C biomass uptake and ^{14}C activity residue for forest (FS), soil treated with CS and RH biochar and without biochar, over a year. Error bars are SEM (n=3). *nd* = not determined

Treatment	Day	Maximum rate /% h^{-1}	^{14}C extents mineralization /%	^{14}C biomass uptake fixed k_{EC} /%	^{14}C activity residue in soil /%
FSC	0	1.79 ±0.11	29.02 ±2.69	14.19 ±1.03	56.78 ±3.66
	60	2.15 ±0.19	49.41 ±5.49	24.77 ±3.61	25.81 ±7.41
	120	2.03 ±0.25	37.10 ±2.11	*nd*	*nd*
	240	1.02 ±0.08	48.55 ±1.73	27.59 ±6.22	23.86 ±7.83
	360	1.03 ±0.30	59.37 ±12.23*	21.73 ±2.31	18.90 ±10.14
FSCS	0	2.06 ±0.19	29.40 ±1.44	14.53 ±2.32	56.07 ±2.04
	60	2.23 ±0.13	44.71 ±3.84	24.33 ±6.45	30.95 ±6.09
	120	2.33 ±0.48	45.01 ±6.05	29.44 ±3.80	25.54 ±4.27
	240	1.54 ±0.19	44.91 ±1.74	26.49 ±3.45	28.59 ±3.62
	360	2.35 ±0.20	83.50 ±8.92*	15.08 ±2.61	1.41 ±6.57
FSRH	0	2.99 ±0.16*	35.68 ±2.22	12.96 ±4.11	51.35 ±2.87
	60	1.65 ±0.17*	58.60 ±4.89	22.81 ±3.79	18.58 ±1.10
	120	2.12 ±0.32*	62.22 ±6.40*	14.31 ±2.33	23.47 ±8.62
	240	1.79 ±0.08*	52.41 ±6.16	28.81 ±5.13	18.78 ±1.56
	360	4.47 ±0.23*	88.79 ±6.86*	12.00 ±1.83	0.00 ±0.00

nd = not determined

Values in asterisk indicate significance at $P<0.05$

Table 3. Maximum rate, ^{14}C extent mineralization, ^{14}C biomass uptake and ^{14}C activity residue for non-intensively farmed (NIF), soil treated with CS and RH biochar and without biochar, over a year. Error bars are SEM (n=3)

Treatment	Day	Maximum rate /% h^{-1}	^{14}C extents mineralization /%	^{14}C biomass uptake (%) fixed k_{EC} /%	^{14}C activity residue in soil /%
NIFC	0	3.34 ±0.52	50.88 ±2.64	5.11 ±0.57	44.00 ±2.09
	60	1.51 ±0.54	29.57 ±10.24	11.49 ±5.09	58.94 ±15.07
	120	2.15 ±0.31	45.70 ±4.07	15.43 ±2.94	38.87 ±5.06
	240	1.96 ±0.10	39.85 ±1.47	15.63 ±0.96	44.52 ±1.83
	360	2.34 ±0.70	48.37 ±7.56*	18.76 ±2.13	32.87 ±6.18
NIFCS	0	3.49 ±0.50	50.28 ±4.83	8.67 ±0.76	41.05 ±4.09
	60	2.00 ±0.79	34.65 ±13.32	6.21 ±1.82	59.14 ±11.65
	120	1.89 ±0.16	40.66 ±1.92	16.87 ±1.01	21.07 ±1.08
	240	2.47 ±0.37	43.90 ±4.47	14.52 ±2.80	41.59 ±4.06
	360	2.51 ±0.33	54.57 ±6.17*	12.24 ±1.94	33.19 ±5.33
NIFRH	0	4.21 ±0.58	47.35 ±3.22	5.36 ±0.94	47.29 ±2.29
	60	1.46 ±0.29	30.19 ±3.80	3.06 ±0.95	66.75 ±4.74
	120	2.13 ±0.20	44.73 ±3.46	9.17 ±3.61	46.10 ±6.84
	240	2.71 ±0.29	48.82 ±3.20	13.87 ±1.10	37.30 ±2.65
	360	4.17 ±0.91	72.77 ±9.37	7.88 ±1.29	19.35 ±8.12

Values in asterisk indicate significance at P<0.05

Table 4. Maximum rate, ^{14}C extent mineralization, ^{14}C biomass uptake and ^{14}C activity residue for intensively-farmed soil (IF), soil treated with CS and RH biochar and without biochar, over a year. Error bars are SEM (n=3)

Treatment	Day	Maximum rate /% h^{-1}	^{14}C extents mineralization /%	^{14}C biomass uptake (%) fixed k_{EC} /%	^{14}C activity residue in soil /%
IFC	0	2.83 ±0.33	44.1 ±1.36	16.29 ±3.67	39.61 ±3.24
	60	1.65 ±0.17	42.7 ±2.94	17.82 ±4.07	39.48 ±5.70
	120	2.12 ±0.32	43.91 ±4.76	14.60 ±1.14	41.49 ±5.51
	240	1.79 ±0.08	36.81 ±1.48	15.44 ±2.31	47.75 ±3.60
	360	3.70 ±0.61	69.80 ±3.51*	14.41 ±1.67	15.78 ±1.88
IFCS	0	3.27 ±0.36	45.79 ±2.51	14.47 ±2.99	39.73 ±2.92
	60	1.83 ±0.07	42.00 ±1.70	12.75 ±3.64	45.24 ±4.89
	120	1.79 ±0.25	40.47 ±3.16	17.13 ±2.24	42.39 ±2.61
	240	2.05 ±0.22	41.49 ±2.97	15.62 ±5.80	42.89 ±8.44
	360	2.81 ±0.77	56.58 ±4.95*	9.67 ±1.82	23.75 ±12.60
IFRH	0	2.98 ±0.72	51.90 ±3.78	9.76 ±3.47	40.24 ±2.15
	60	1.58 ±0.29	41.23 ±2.75	18.00 ±3.58	40.77 ±1.21
	120	1.57 ±0.19	38.42 ±3.93	19.48 ±3.57	42.10 ±1.41
	240	1.54 ±0.07	31.92 ±1.90	15.26 ±1.15	52.82 ±2.76
	360	2.40 ±0.58	51.77 ±6.45*	13.51 ±1.06	34.72 ±5.41

Values in asterisk indicate significance at P<0.05

Adding either biochar had no effect on the ^{14}C mineralization and ^{14}C-glucose incorporation into the microbial biomass, except after 360 days of incubation in forest soil, where there was enhanced ^{14}C-glucose mineralization (Figure 1a and Table 2). Here, after 360 days of incubation, CS and RH biochar-amended soils had an average of 83% and 88% mineralization, respectively compared to forest control soil (59%). Owing to high levels of ^{14}C-glucose availability and mineralization in these amendments, the remnant ^{14}C-glucose fractions were taken up into the biomass and did not remain as residual carbon, when compared to other soils and control (Tables 2-4). Mineralization of ^{14}C-glucose accelerated due to the high carbon content as a result of RH biochar addition to the soil in comparison with the soil with no biochar. Unlike the forest soil, the intensively-farmed soil and non-intensively farmed soil displayed the reverse trend of biomass uptake and mineralization. For example, the mineralization of ^{14}C-glucose in the intensively-farmed soil was greater than that in the forest soil (44.1% and

29.02%, respectively) at the earlier time of incubation (Figures 2a and c; and Tables 2 and 4). However, the opposite pattern occurred towards the end of the incubation period. Mineralization of ^{14}C-glucose in the forest soil, amended with RH biochar (88.79%), (Table 2) was higher than in the intensively-farmed soil (51.77%) amended with the same biochar (Figure 1a and; Tables 2 and 4). Due to the high sand content in the forest soil, the opportunity for the biochar-soil colloid bridge interaction was limited. With this limitation, 14C-glucose availability was reduced. Uptake by the microbial population in the forest soil similarly declined, in contrast to the intensively-farmed soil (12% and 13.51%, respectively) (Tables 2 and 4). Mai et al. (2020) showed that biochars produced under oxygen exposure had high contents of soluble salts, which ensured bridging between soil colloids through increased ionic strength and multivalent cations. When such interaction occurs, a significant amount of glucose can then be adsorbed onto and within the biochar-soil matrix (Giovanna et al. 2017). Hence, biochar and soil interactive properties are of prime importance to influence labile carbon availability and microbial activity.

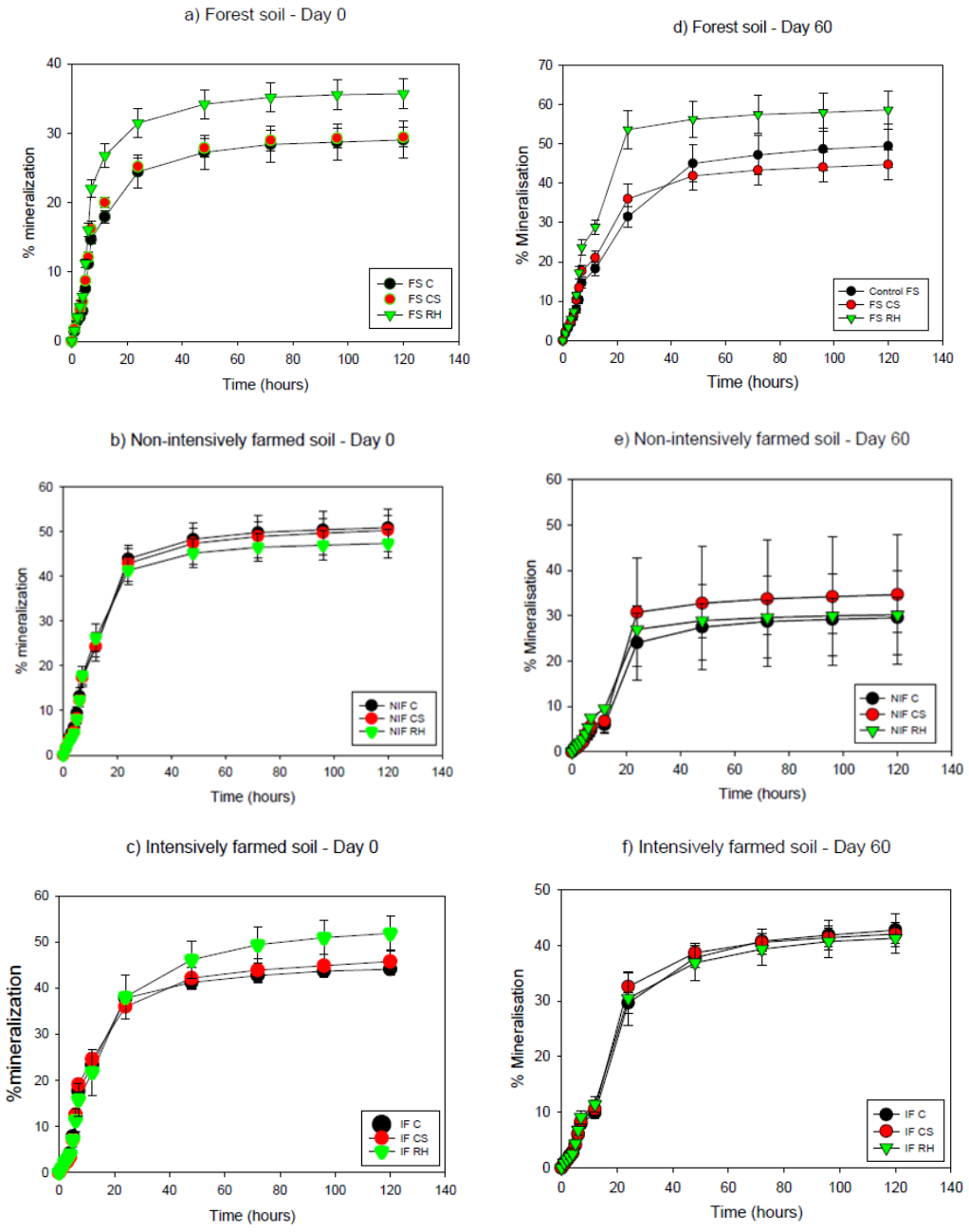


Figure 2. Mineralization of ^{14}C-glucose on days 0 and 60 of the; a), d) forest; b), e) non- intensively farmed; and c), f) intensively-farmed soils amended with CS and RH biochar and without biochar. Error bars are SEM (n=3)

3.4 Effect of Biochar on Ammonium Ion Losses via Leaching

There were decreases in ammonium ion leachate in all soils over incubation period (Figures 3a, b and c). However, this relationship varied between soils. For instance, as leaching commenced, biochar amendment ensured decreased ammonium ion leachate in the forest and non-intensively-farmed soil from 0.03 mg/L in control to 0.00067 (CS) and 0.003 (RH) mg/L ($P<0.05$) (Figure 3b). Meanwhile, in the intensively-farmed soil, there was insignificant effect on ammonium leaching following biochar amendment (Figure 3c). Results have also shown that in the forest soil control treatment, ammonium leaching was higher than in the biochar treatment. The biochar held ammonium ions in the forest soil over the course of the study ($P<0.05$) owing to the presence of negative charges on the biochar surface (Novak et al. 2009), which independently enables ionic interaction with positively-charged cations like ammonium (Wong et al. 2019).

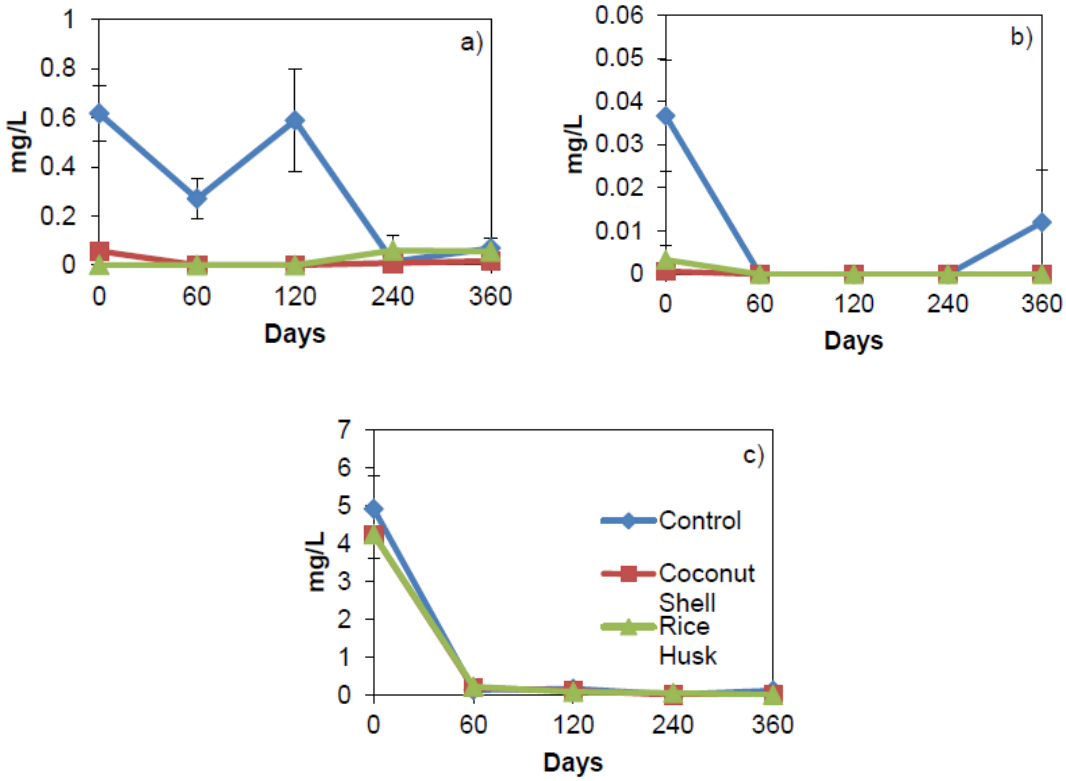


Figure 3. Concentration of ammonium over a year in the leachate of the a) forest, b) non- intensively farmed, and c) intensively farmed soils treated, with CS and RH biochar and without biochar. Error bars are SEM (n=3)

3.5 Effect of Biochar on Nitrate Ion Losses via Leaching

Nitrate leaching patterns were different amongst all soils studied. Compared to ammonium ions, the amount of nitrate ions in the forest soil leachate was higher over the course of the experiment ($P<0.05$). Meanwhile in the non-intensive and intensively-farmed soils, the concentration of nitrate ions decreased over time ($P<0.05$) (Figures 4a, b and c). Amending the soils with biochar did not produce significant differences in nitrate concentrations ($P>0.05$) in leachate (Figures 4a, b and c). In the forest soil, nitrate leachate concentration increased consistently to approximately 20 mg/L irrespective of biochar type or without biochar. Interestingly, biochar addition initially retarded nitrate leaching before 60 days (short-term) when compared to the control soil (Figure 4a), but as contact time increased, the limit for biochar to encourage nitrate retention was exceeded and leaching occurred as in the control. The forest soil exhibited lower pH and higher CEC compared to the other soils, but these properties were insufficient to limit nitrate leaching. The influence of the well-drained sandy feature of forest soils obviously accelerated leaching of nitrates (Shrestha et al., 2010; Fraters et al. 2015). In addition to the sandy soil texture, the C/N ratio (19) of the forest soil was much higher than those of other soils. This condition is highly suitable for degradation of organic nitrogen through nitrification. Apparently, the types of biochar used in this study were incapable to limit leaching in the soils for a longer time. Similarly, Ghorbani et al. (2019) reported that soils having higher clay contents amended with biochar exhibited much lower nitrate

leaching compared to sandy soils. More recently, Lv et al. (2021) showed that rice straw biochar could not retard nitrate losses through the leaching process. Nevertheless, since biochars contained higher nitrogen contents than the soils studied, the data observed does not rule out nitrate release directly from biochar following oxidation. Yet, nitrate leachates decreased over time in the non-intensive and intensively farming soils. It is possible that this decrease is due to the leaching of nitrates derived from previous fertilization of the soil which was not replenished.

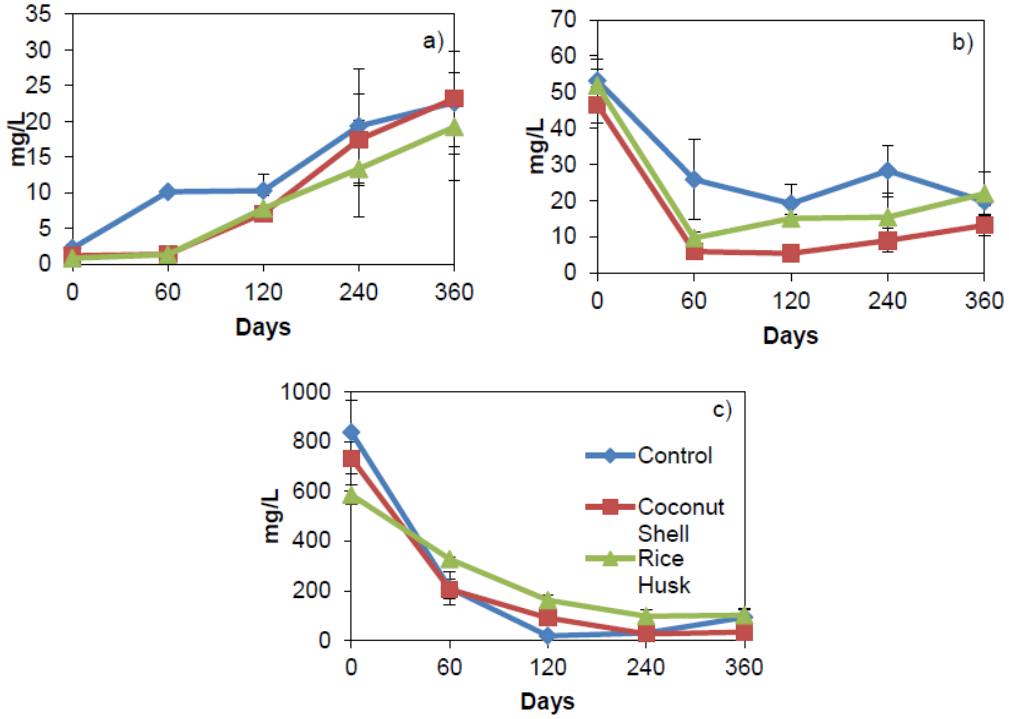


Figure 4. Concentration of nitrate over a year in the leachate of a) forest, b) non- intensively farmed, and c) intensively farmed soils, treated with CS and RH biochar and without biochar. Error bars are SEM (n=3)

3.6 Effect of Biochar on Phosphate Ion Losses via Leaching

The concentration of phosphate ions leached displayed different trends in all of the soils studied. For example, the amount of phosphate ions leached from the forest soil was low when compared to the intensively-farmed soil leachate. The phosphate concentration in the forest soil leachate was not constant over time and possibly close to the limits of detection, but in the non-intensively farmed and in the intensively-farmed soils the phosphate concentrations in the leachate declined over the course of the study. Likewise, the phosphate concentration in the leachate of both soils showed a little increase at the end of the leaching experiment (Figures 5a, b and c). The decline in phosphate leaching in these soils was probably due to the leaching of previously applied fertilizer P. Amending the forest soil with biochar insignificantly decreased ($P>0.05$) phosphate leaching, whilst amending the non-intensively farmed soil with the RH biochar had greater phosphate concentration in the leachate ($P<0.05$) at the beginning of leaching exercise due to higher P content in the RH biochar (Figure 5b).

The reduction in phosphate leaching in the forest soil was not just due to biochar, but other soil properties could influence it, such as the levels of iron and aluminum contents influence phosphate sorption in lower pH (<5) soils (Barrow 2017). In addition, biochars can only adsorb a minimal amount of phosphate in soils (Singh et al. 2010) due to a low anion exchange capacity. Further, Yao et al. (2012), observed that five out of thirteen different biochars amended in sandy soils adsorbed phosphate, whilst there was phosphate leachate release in the remaining biochars. Furthermore, results revealed that biochar produced hydrothermally leached the greatest amounts of nitrate and phosphate ions. Also more than 2% phosphate was leached from three types of bamboo biochar. In the present study, phosphate leaching was high from the soils amended with RH biochar. This was due to the fact that the biochar itself has a high phosphate content (1.75mg g^{-1}) (Table 1). Additionally, the phosphate content of the intensively-farmed soil was relatively high due to the addition of large quantities of fertilizer to the soil.

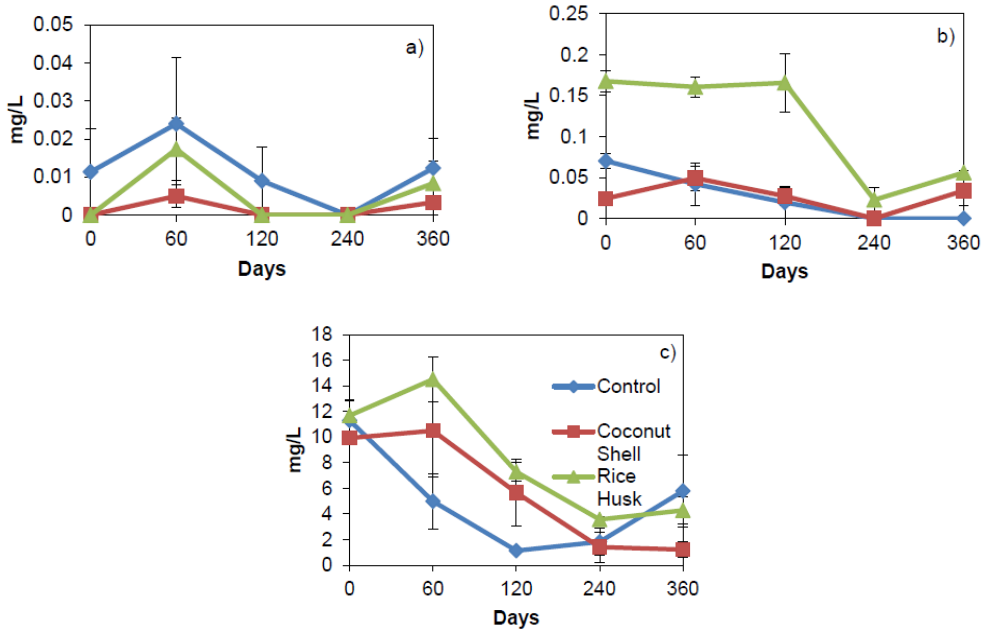


Figure 5. Concentration of phosphate over a year in the leachate of a) forest, b) non- intensively farmed, and c) intensively-farmed soils, treated with CS and RH biochar and without biochar. Error bars are SEM (n=3)

4. Conclusions

In conclusion, adding biochars (CS and RH) to tropical soil exhibited a minimal outcome in this study. The biochar addition increased the pH and carbon, and reduced ammonium leaching of forest soil. Application of biochars also had marginal effects on the CEC and microbial activity. However, biochar had no effects on the other soil characteristics, such as nitrogen and phosphate contents in the soil, as well as microbial biomass. Nevertheless, successful deployment of biochar in soils, strongly depends on the properties of the biochar and soil. Although the biochar additions had mostly a neutral effect on the soils, the positive effect on soil pH is worthy of explorations at a bigger scale, for instance in a field test, to evaluate whether the effects persist in natural conditions. Lime is costly in tropical countries and biochar may provide a useful alternative for pH management. In addition, further studies are required to evaluate the influence of such biochar on the fate of heavy metals in soil. Also, investigations on optimizing the pyrolysis conditions for enhanced agronomic properties can be explored.

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Authors contributions

Author Khasifah Muhamad contributed to the material preparation, data collection and analysis. The first draft of the manuscript was written by Khasifah Muhamad. The manuscript was commented by Prof. John Quinton and Prof. Kirk Semple. The revised draft of the manuscript was written by Khasifah Muhamad. Author Dr Uchenna Ogbonnaya written and revised the final draft of manuscript. All authors read and approved the final manuscript.

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Competing interests

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.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

Not commissioned; externally double-blind peer reviewed.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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