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Effect of Varying Biochar Particle Sizes and Concentrations on Soil Nutrient Retention and Microbial Activity

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ABSTRACT

This study aims to determine the effect of adding biochar to soil under different management systems, as well as soil nutrient availability in a temperate environment. We tested whether biochar could enhance the chemical and biological properties of soil and reduce nutrient leaching. There were two parts of the study. These two studies were not related with each other, but the only similar study approach was the ageing effect of biochar (incubated of soil mixture for up to 300 days and 30 days). In the first part of the study, 2% of biochar by weight with a <5 mm particle size was produced from hardwood and incorporated into three different types of soil. The three types are an arable loam soil, an arable sandy soil and a grassland soil. The soils with and without biochar (control) were incubated for up to 300 days. In the second part of the study, different dosages of hardwood biochar (2% and 5%) with various particle sizes (2, 1, 0.5 and 0.1 mm) were incorporated into soils with different nutrient status (fertilised and unfertilised soils) and incubated for up to 30 days. The findings from the study exhibited that hardwood biochar significantly increased the mineralisation of ^{14}C-glucose at 5% biochar dosage and at finer particle size. The pH of soil and carbon and the microbial biomass in unfertilised soil also increased after biochar addition. Adding biochar to soil had no major change on the ageing effect of the biochar and the leaching of nitrate ions, but reduced the ammonium ion leaching. The efficacy of biochar application depends on soil type, nutrient availability, biochar application rate and particle size.

Keywords: Temperate soil, Biochar, Carbon, Leaching, Mineralization

1. Introduction

Research suggests that adding rich organic material with recalcitrant carbon (C) results in increased C content in the soil (Zhang et al. 2017). Charred organic matter usually contains significant levels of recalcitrant C resistant to microbial degradation and can reside in soils for a long time, whilst influencing soil properties that improve the agronomy (Lehmann et al. 2003). Terra preta soils have demonstrated the potential to improve and sustain soil fertility by interacting with inherent colloids, nutrients and soil organic carbon to create conditions that lead to sustainability (Busch & Glaser 2015). This is of particular importance to soil due to the trends in degradation and the sensitivity of extractable organic matter and nutrients (Chen et al. 2018). The sustainability of soil fertility through agronomic practices has been a challenge to scientists owing to changing climatic conditions, changes in land use, and the availability and applicability of soil amendment measures (Paroissien et al. 2015). It has been estimated that an average of 60% of nitrogen that is applied to soils for crop production globally is either leached or undergoes surface runoff due to irrigation, whilst only the residual fraction is utilized by crops (Zhou et al. 2019). Hence, improving crop nutrient utilization rates and eliminating nutrient loss following irrigation regimes is essential (Zhou et al. 2019).

The efficacy of several soil amendments to alleviate nutrient leaching to the environment has been tested, either alone or in mixtures (Wang et al. 2016). Biochar, a form of soil amendment, can readily be made from various organic materials, such as wood, food waste, plant debris by pyrolysis. The resistant aromatic C structure of biochar supports soil aeration, aggregation, water holding capacity, compaction resistance and slow decomposition of soil carbon (Wong et al. 2019). Naturally, pyrogenic carbon in top soils represents the highly stabilized and recalcitrant carbon content of soils (Dynarski et al. 2020). Hence, the systematic application of biochar as a stable organic carbon moiety into agricultural soils can contribute to the naturally-stabilized organic carbon sinks. Due to the biochar's potential as a soil ameliorant, this study tests fertility increases in well-managed soils amended with biochar. How particle size and dosage of biochar influence the efficacy of biological activity and reduction of soil nutrient leaching were also investigated. Our hypotheses are that biochar will greatly improve the fertility of unfertilised soil to

a greater extent than fertilised soil. The finer particles of biochar will hold more nutrients than the coarser particles and 5% of biochar application will enhance the quality of soils in both fertilised and unfertilised soils compared to the lower dosage (2% application rate of biochar).

2. Material and Methods

This research consisted of two sets of experiments. The soils were collected from two sites in the UK and the biochar used in these studies were similar.

2.1. Chemicals and biochar

^{14}C glucose was obtained from Sigma Aldrich Co. Ltd. UK. Goldstar multipurpose liquid scintillation cocktail, carbo trap and carbo count were obtained from Meridian, UK. Combust aid was obtained from Perkin Elmer, USA, while the chloroform ($CHCl_3$), potassium sulphate and sodium hydroxide were supplied by Fisher Scientific, UK.

The biochar used in this study was the combinations of hardwoods which are primarily beech (Fagus spp.), and to a lesser extent ash (Fraxinus excelsior), oak (Quercus spp.), birch (Betula spp.) and cherry (Prunus spp.). Biochar was obtained from (Bodfari Environmental, St. Asaph, UK). Harwood biochar was chosen from the source because such biochar exhibit higher surface area, pore volume, liming effect and cation exchange capacity than most other biochar feedstocks (Jiang et al. 2017; Ippolito et al. 2020). Such properties are critical for nutrient retention. The method by which this biochar was produced was slow pyrolysis for 24 hours in a ring-kiln at 400 °C. Some of the biochar characterisation was conducted in the similar method with the soil characterisation. The properties of biochar used in these experiments are shown in Table 1.

2.2. Soils

Soils were collected from five various locations with similar environmental conditions, depths, as well as similar soil texture, but in the United Kingdom. However, three of the soils were from the same local area (Dundee), whilst two were from another site (Penrith), thus divided into two experimental conditions. Nevertheless, all soils were incubated with the same biochar at different conditions to investigate substrate respiration and leachability.

2.3. Experiment 1

Arable loam, arable sandy and grassland (Brown Earth) soils from Dundee, United Kingdom were collected. An arable sandy and an arable sandy loam – both in crop rotation, whereas a grassland soil was from managed, perennially grassed land. All soils were obtained from the top 20 cm of the soil profile, from the James Hutton Institute in Dundee, UK (56° 27’ N, 3° 4’ W, 29 m.a.s.l). To study the effects of aged biochar associated in soil, the biochar was added and mixed into the soils and left several months in the pots (approximately 10 months). Pots had a capacity of 38 L, with dimensions of 38 x 38 x 30 cm. Soils were mixed and placed into pots and biochar was sieved to remove particles > 2 cm in size, and mixed with half of each soil type equivalent to 2.0% of soil dry weight. Soil that did not contain biochar was also added in the pot as a control treatment. The wet-weight of each pot was approximately 25.2 kg dry soil, thus 2% biochar-treated weighed 25.7 kg total substrate. Soil or soil-biochar mix was added to pots in four equal portions and compacted by hand between each addition to ensure similar compaction.

Approximately 10 months later, a 15-cm cylindrical core was used to collect the mixture (biochar + soil) and non-mixture (no biochar) of soil samples from the pot. Composited soil samples were obtained from 4 random points in each pot. In the laboratory, the samples (soil from non-biochar treatment pots) were mixed with 2% of fresh hardwood (HW) biochar by weight, with < 5 mm particle size in thriplicate. This is to differentiate the aging effects of previously added biochar in the pot, with fresly added biochar in the laboratory. Whereas, soil with no biochar acted as a control treatment with three replications. All soil samples were then incubated for 0, 60, 180, and 300 days and kept in screw-capped jars. At each contact time and before analysis, soils were dried and sieved with a 2-mm mesh for soil aggregates suitable for soil analysis (Kandeler 2007). Table 2 displayed soil characteristics determined in this study.

2.4. Experiment 2

In this experiment, brown earth soils were collected from Penrith, Cumbria UK. These soils were chosen based on differences in soil management. The first soil was from oilseed rape-area which was properly managed i.e. well-fertilised. The second soil was collected from grassland which was not properly managed. No fertiliser had been applied for approximately 50 years. The texture of all soils was sandy clay loam. The soil samples were obtained with a shovel from the field to a depth of up to 15 cm. The soil samples were sieved through a 5-mm mesh, and amended with 2% and 5% of HW biochar with various particle sizes (2, 1, 0.5 and 0.1 mm). Unamended soil acted as a control treatment. The samples were placed in screw-capped glass jars and incubated for a month. The amounts of soil samples placed in each jar was estimated based on the total chemical and biological analyses tests. 2% and 5% of HW biochar with different parrticle sizes were then added in relative to the weight of the soil in

each jar. Lastly, soils were dried and sieved with a 2-mm mesh to achieve appropriate soil aggregates for the soil biological and chemical analyses (Kandeler 2007). The soil's physical and chemical properties are presented in Table 2.

2.5. Biological and chemical analyses of soil

Substrate-induced respiration was employed for the biological respirometry experiments (Reid et al. 2001). This experiment was carried out in order to measure the activity of microbes in the soil (respiration and biomass). Therefore, ^{14}C-glucose is used as a carbon source for microbes to utilise for respiration (release of $^{14}CO_2$) and ^{14}C- carbon incorporation into microbial biomass (Boucard et al. 2008). To assess the microbial respiration and biomass, the methods are explained as follows.

In experiment 1, at 0 and 60 days contact time, 20 g wet weight of soil was collected from jars and added to respirometry flasks with 3 mM ^{14}C-glucose solution (radioactivity of 1086 Bq) added to the soil samples, whilst 654 Bq glucose was used on days 180 and 300 (contact time). Meanwhile, in experiment 2, 3 mM of glucose solution was added to the soil samples, with radioactivity of 1051 Bq on days 0 and 30 (contact time). Both experiments followed a modified method of Doick & Semple (2003). Then, a 7-mL vial containing 1 mL NaOH (1 M) solution CO_2 trap was suspended from the lid of each respirator for the glucose mineralization. Samples were then shaken on an orbital shaker at 100 rpm. The rates of ^{14}C-glucose mineralization were measured hourly within four hours, then every two hours for another four hours in 24 hours. Rates of mineralization were also measured each day within 5 days. During the period of sampling, the NaOH vial was removed and well wiped with blue roll tissue soaked in acetone to dissolve residual ^{14}C on the surface. Afterwards, 5 mL of liquid scintillant cocktail was added into the vial and incubated in the dark overnight prior to measuring ^{14}C-activity using a liquid scintillation analyser (Canberra Packard Tri-Carb 2250A).

To determine the biomass, 4 g of soil slurry from the respirometer was collected on the last substrate sampling day, when extraction of non-fumigation sample was done with 20 mL of 0.5 M K_2SO_4. The samples collected were shaken on an orbital shaker for 30 minutes at 100 rpm, the supernatant was filtered and an aliquot (5 mL) of the supernatant was incorporated into a 20-mL vial. For liquid scintillation counting, 15 mL of liquid scintillant cocktail was also added into each vial and the samples were kept in the dark overnight. Simultaneously, the remaining samples were fumigated using a desiccator lined with soaked blue roll tissue, whilst at the centre of the desiccator, 75 mL of ethanol-free chloroform ($CHCl_3$) was placed. After vigorous boiling of $CHCl_3$ within 2 minutes, the desiccator was evacuated. After 24 hours, repeated five- or six-fold evacuation was done to ensure residual $CHCl_3$ vapour was removed. Subsequently, the soils were similarly extracted and counted as with the non-fumigation extraction method. The amounts of ^{14}C left in the soil treatments were assessed through dry combustion of 1 g of soil. The associated residual ^{14}C-glucose activity of the soils was then determined via a 3-minute combustion on the Packard, Model 307 sample oxidiser.

For chemical soil analyses, 10 g of air dried soil was used to analyze pH. pH was then measured using a pH meter model PHM 220 which was calibrated using buffers at pH 4.0 and 7.0. In addition, samples of approximately 30 mg were used for determination of total carbon. Total carbon (C) was determined by dry combustion and measured with an elemental analyser (Elementar Vario EL).

2.6. Nutrient leaching (experiments 1 and 2)

In experiment 1, the hardwood biochar that was previously added in the arable loam, arable sandy soil and grassland soil for more than 10 months was used, as well as freshly-added biochar amended in the same types of soil. Soils with no biochar acted as controls. Soils with and without biochar (2%; <5 mm particle size) were adjusted to a 1.2 g cm^{-3} bulk density and packed into 27 PVC columns in triplicate. Each column has a diameter of 5 cm and is 20 cm long. Subsequently, at the bottom of the PVC column, 40 g of sand was poured to avoid the clay particles from being lost during the leaching experiment. At the end of the column used, two layers of nylon mesh were placed, lined and secured with cable ties.

In experiment 2, hardwood biochar with various particle sizes i.e. 2, 1, 0.5 and 0.1 mm were used. The samples of fertilised and unfertilised soils were amended at two different application rates (2% and 5%). Soils amended with biochar and un-amended soil were adjusted to approximately 1.2 g cm^{-3} bulk density. Then the samples were filled into a glass column of diameter 5 cm and length 20 cm. Due to the lack of glass columns, the samples were in duplicate. All of the leaching processes commenced by pouring 100 mL of deionised water through each of the soil columns. An Erlenmeyer flask was used to collect leachates and then stored at 4 ^{O}C for two to three days before analysis. Ammonia and nitrate leachates were analysed using a Bran + Luebbe Autoanalyzer 3.

2.7. Analyses of statistics

The mean values of maximum rate, ^{14}C mineralization, ^{14}C biomass, pH, total carbon and leachate nutrient concentration amongst treatments and during contact time, were tested using a one-way analysis of variance (ANOVA) ($P<0.05$). The Holm-Sidek procedure ($P<0.05$) was used for comparison of multiple means. Statistically insignificant values were further tested by the non-parametric Kruskal-Wallis test based on ranks. In addition, the significant differences amongst treatments for non-distributed

values were tested by Tukey's test ($P<0.05$). All statistical tests were performed using the SigmaStat v. 3.5 (Systat Software Inc.).

3. Results and Discussion

3.1. Soil chemical characteristics (experiments 1 and 2)

The carbon contents of amended grassland, arable loam and arable sandy soil with fresh and aged biochar samples increased (Figures 1a, b and c). Also, fresh and aged biochar application to soil significantly increased the soil pH ($P<0.05$) (Figures 1d, e and f). This is due to the high pH of biochar (9.05) used in this study (Table 2).

Furthermore, the results also show that the smaller sizes (1, 0.5 and 0.1 mm) of biochar increased C content ($P<0.05$) more than the coarser particle sizes (2 mm) and in the treatment with no biochar (Figures 2a and b). This is because of the higher tendency of biochar to interact with soil microbes, as well as soil organic matter (de Jesus Duarte et al. 2019). In the meantime, the smaller particle sizes (0.5 and 0.1 mm) had the greatest value of soil pH ($P<0.05$) in contrast to the bigger particle sizes (1 and 2 mm) and control (Figures 3a and b). This is due to the bigger surface area of the smaller particle size of biochar.

Table 1- Biochar chemical characteristics

Parameter	***Hardwood Biochar***
% Carbon	71.38
% Nitrogen	0.45
C/N Ratio	158.68
CEC (meq 100 g^{-1})	34.36
pH	9.05
Inorganic P (mg g^{-1})	0.41

Table 2- Soils physical and chemical characteristics

Parameter	***Experiment 1***			***Experiment 2***	
	Grassland	*Arable Loam*	*Arable Sandy*	*Fertilised*	*Unfertilised*
% Clay	34.90	34.41	27.54	28.29	28.12
% Silt	17.44	16.06	6.36	11.96	9.00
% Sand	47.67	49.53	66.10	59.75	62.88
Texture	Sandy Clay	Sandy Clay Loam	Sandy Clay Loam	Sandy Clay Loam	Sandy Clay Loam
% Carbon	2.39	3.79	2.06	2.14	3.40
% Nitrogen	0.14	0.21	0.16	0.19	0.19
C/N Ratio	16.45	17.75	12.68	11.26	17.89
CEC (meq 100 g^{-1})	13.49	13.86	9.24	-	-
pH	6.08	6.53	5.81	6.16	6.15
Inorganic P (mg g^{-1})	1.15	1.57	1.15	-	-

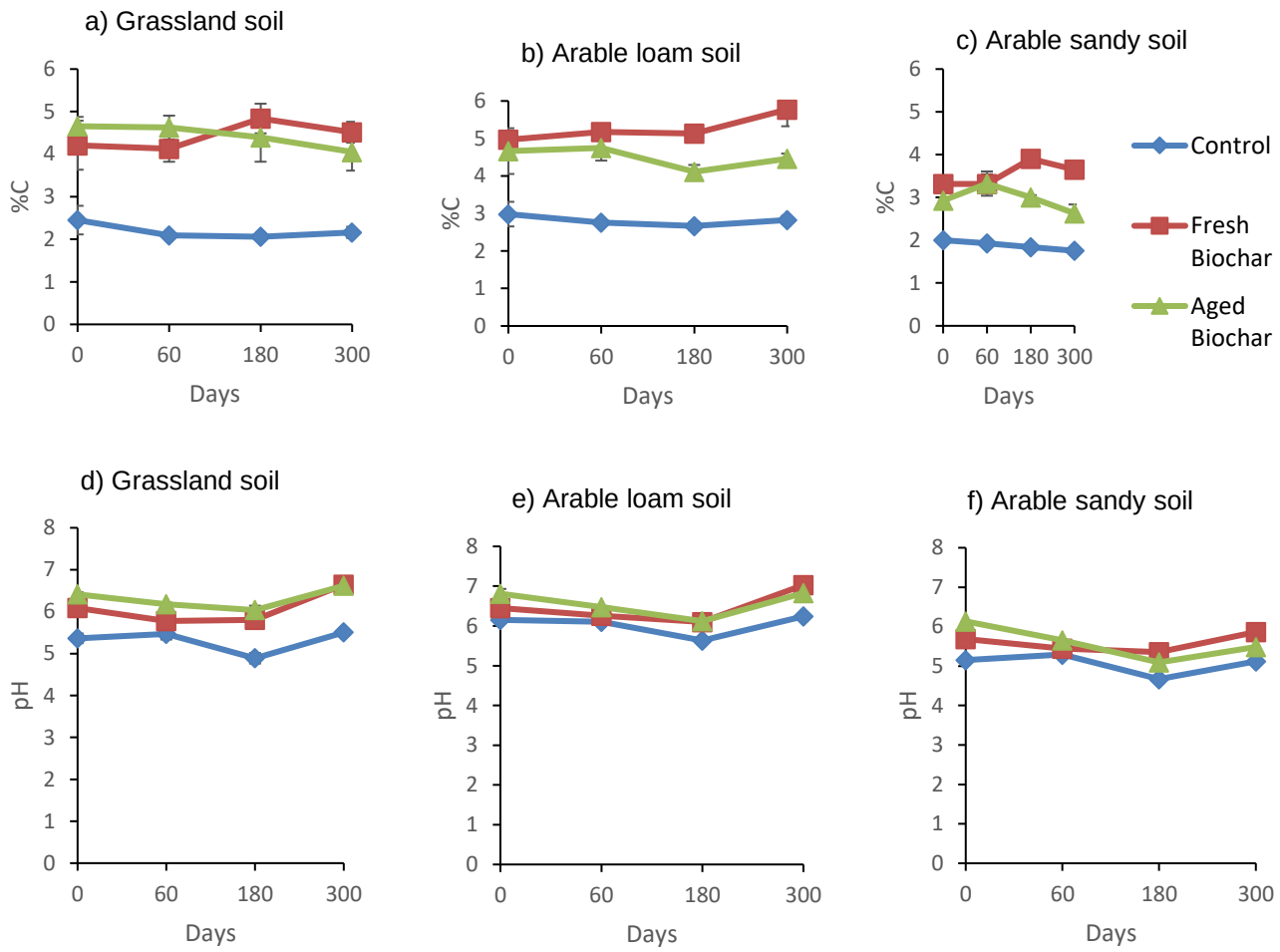

Figure 1- The carbon and pH content in the a) and d) grassland, b) and e) arable loam and c) and f) arable sandy soils treated with fresh and aged biochar; and with no biochar over 300 days. Error bars are SEM (n=3)

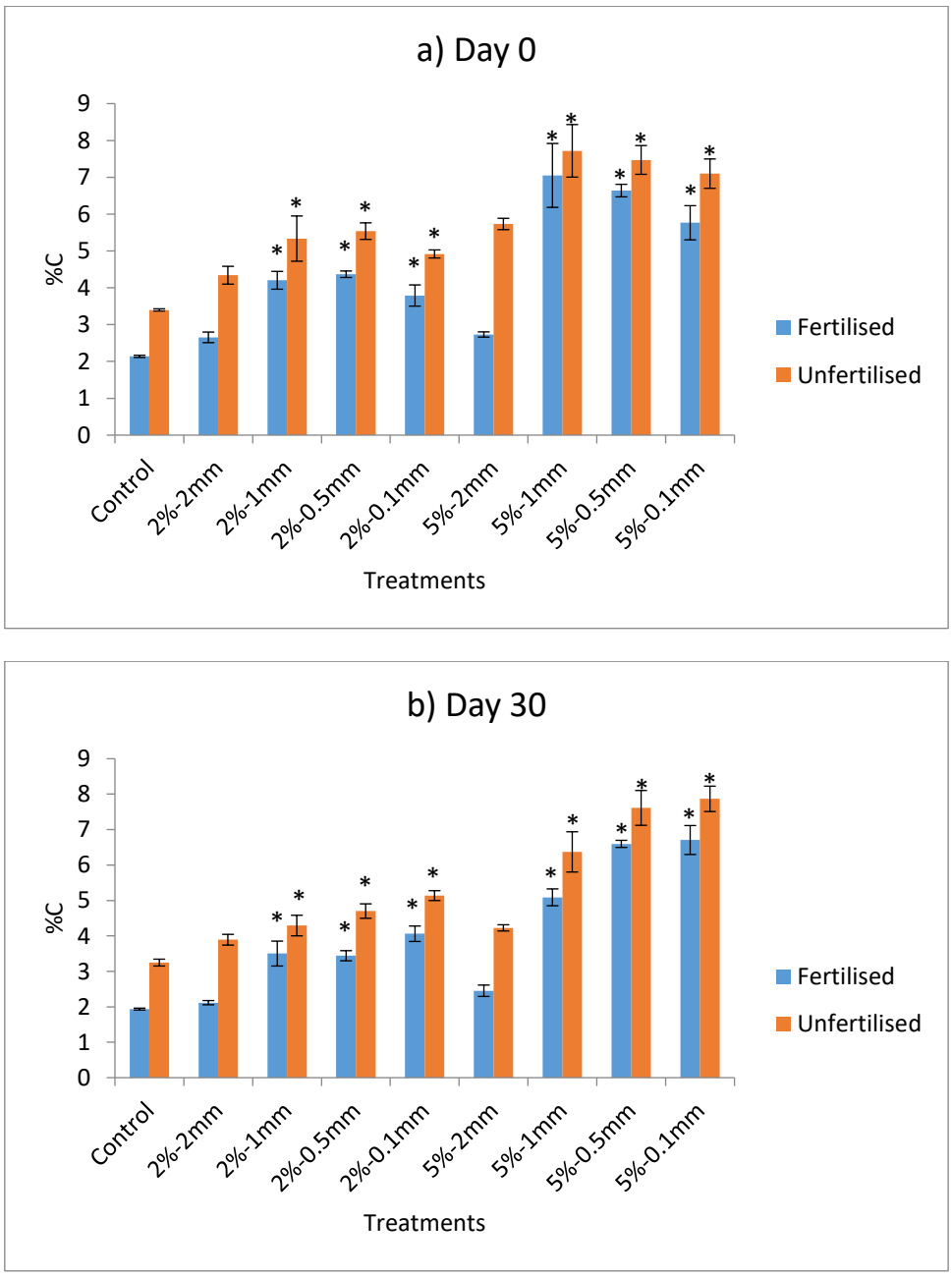

Figure 2- Carbon in fertilised and unfertilised soils in the a) Day 0 and b) Day 30, treated with 2% and 5% of biochar; and no biochar (control). Error bars are SEM (n=3). Values in asterisk indicate significance at P<0.05

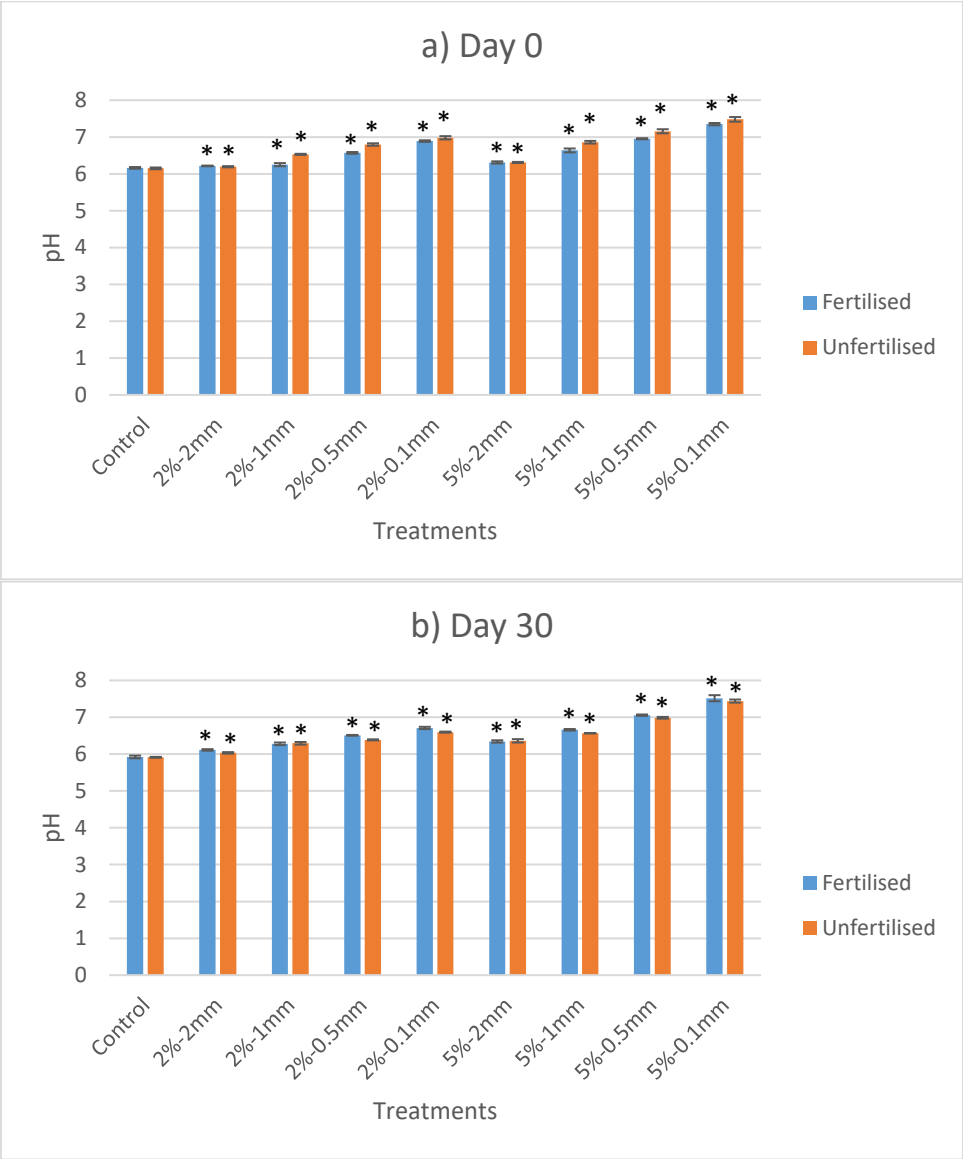

Figure 3- pH in fertilised and unfertilised soils in the a) Day 0 and b) Day 30, treated with 2% and 5% of biochar; and no biochar (control). Error bars are SEM (n=3). Values in asterisk indicate significance at P<0.05

3.2. Mineralisation of ^{14}C-glucose to $^{14}CO_2$ and incorporation of ^{14}C-glucose into microbial biomass

3.2.1. Experiment 1

During the period of incubation, the extents of mineralisation of ^{14}C-glucose in the three contrasting soils (grassland, arable loam and arable sandy soils) were constant. In addition, fresh and aged biochar application to soils did not show any major change during the incubation time. Just after 180 days of incubation it was seen that the extent of ^{14}C-glucose mineralisation in grassland soil amended with the aged biochar (74.86%) was significantly higher ($P<0.05$) than in the treatment of fresh biochar-amended soils (62.83%) (Figure 4c and Table 1). The maximum rates of ^{14}C-glucose mineralization were observed to have no significant differences in the arable loam and arable sandy soils (Tables 2 and 3). The significant effect on the maximum rates was only observed on day 180 in the aged biochar incorporated in grassland soils (4.46% h^{-1}), in comparison with the rates of fresh biochar (3.51% h^{-1}) and with no biochar (3.80% h^{-1}) in the same soil (Table 1).

Table 1- Maximum rate, ^{14}C extent mineralisation, ^{14}C biomass uptake and ^{14}C activity remaining for grassland soil amended with and without fresh biochar (FB); and aged biochar (AB), over 300 days. Error bars are SEM (n=3)

Treatment	Day	Maximum rate (% h^{-1})	^{14}C extents mineralisation (%)	^{14}C biomass uptake (%) fixed k_{EC}	^{14}C activity remaining in soil (%)
Grassland	0	2.32 ± 0.15	42.54 ± 2.46	7.99 ± 0.54	49.47 ± 2.46
Control	60	3.68 ± 0.28	75.91 ± 2.61	20.28 ± 5.07	3.81 ± 4.61
	180	3.80 ± 0.15	67.18 ± 0.45	11.32 ± 0.24	21.49 ± 0.35
	300	3.39 ± 0.11	76.39 ± 3.99*	20.77 ± 1.89	2.83 ± 4.82
FB	0	2.35 ± 0.24	41.37 ± 3.09	10.73 ± 2.25	47.90 ± 3.92
	60	3.43 ± 0.49	71.95 ± 5.28	25.30 ± 3.51	2.74 ± 6.26
	180	3.51 ± 0.20	62.83 ± 2.24	11.02 ± 0.90	26.15 ± 1.35
	300	2.84 ± 0.21	71.14 ± 2.74*	25.20 ± 2.56*	3.66 ± 4.45
AB	0	2.17 ± 0.43	42.72 ± 5.27	12.96 ± 4.21	44.31 ± 2.67
	60	3.21 ± 0.40	75.44 ± 5.17	22.73 ± 0.06	1.83 ± 5.18
	180	4.46 ± 0.20*	74.86 ± 2.07*	9.77 ± 1.75	15.37 ± 1.68
	300	5.37 ± 1.30	93.59 ± 16.09*	11.36 ± 2.90	0.00 ± 0.00

Values in asterisk indicate significance at P<0.05

Generally, biochar addition to soil did not have a great effect on the microbial biomass in the soil. On day 0, the uptake of ^{14}C-carbon into the microbial biomass in arable loam soil amended with aged biochar was significantly higher (P<0.05) than fresh biochar amended with the same soil (12.56% and 7.68%, respectively) (Table 2). However, after 60 days of incubation, the mineralisation of ^{14}C-carbon in arable loam soil amended with aged biochar was significantly (P<0.05) higher (88.12%) in comparison with the fresh biochar-amended soil (68.33%), and in the treatment with no biochar in the same soil (69.91%) (Figure 4f and Table 2). Furthermore, incorporation of ^{14}C-carbon into microbial biomass after 300 days contact time showed higher uptake in grassland soil amended with fresh biochar (25.20%) (P<0.05) compared to the uptake in the same soil amended with the aged biochar (11.36%) (Table 1). Also, the maximum rates were higher in the treatment with no biochar (control) (4.77 h^{-1}), then in the treatment with fresh biochar (3.00 h^{-1}) and aged biochar (3.44 h^{-1}) after 300 days of incubation in arable loam soil (P>0.05) (Table 2).

Table 2- Maximum rate, ^{14}C extent mineralisation, ^{14}C biomass uptake and ^{14}C activity remaining for arable loam soil amended with and without fresh biochar (FB); and aged biochar (AB), over 300 days. Error bars are SEM (n=3).

Treatment	Day	Maximum rate (% h^{-1})	^{14}C extents mineralisation (%)	^{14}C biomass uptake (%) fixed k_{EC}	^{14}C activity remaining in soil (%)
Arable loam	0	3.08 ± 0.58	54.76 ± 7.56	11.23 ± 0.57	34.01 ± 7.23
Control	60	3.09 ± 0.22	69.91 ± 3.25	21.00 ± 3.03	9.09 ± 5.87
	180	4.01 ± 0.21	71.94 ± 2.75	8.72 ± 0.41	19.34 ± 2.42
	300	4.77 ± 0.77	85.66 ± 10.72*	15.15 ± 1.68	0.00 ± 0.00
FB	0	2.80 ± 0.31	52.47 ± 4.24	7.68 ± 0.19	39.85 ± 4.43
	60	2.88 ± 0.32	68.33 ± 0.82	19.74 ± 2.33	11.93 ± 2.05
	180	3.39 ± 0.24	63.99 ± 3.75	10.05 ± 1.27	25.95 ± 4.95
	300	3.00 ± 0.14	69.88 ± 0.72*	18.19 ± 1.92	11.91 ± 2.47
AB	0	2.24 ± 0.05	46.94 ± 1.35	12.56 ± 0.09*	40.49 ± 1.44
	60	3.79 ± 0.47	88.12 ± 7.15*	18.61 ± 1.91	0.00 ± 0.00
	180	3.76 ± 0.12	70.29 ± 0.55	8.96 ± 0.09	20.75 ± 0.48
	300	3.44 ± 0.18	75.52 ± 2.93	15.68 ± 2.21	8.81 ± 1.53

Values in asterisk indicate significance at P<0.05

The variations in the extent of ^{14}C-glucose mineralisation had a small effect in the soil amended with both fresh and aged biochar. The major change only occurred after 60 and 180 days, when the aged biochar increased the extent of ^{14}C-glucose mineralisation in the arable loam and grassland soils (Figures 4f and c). Whereas, the extent of ^{14}C-glucose mineralisation in the arable sandy soil demonstrated significant effect only on the final period of incubation (day 300) (Figure 4l and Table 3). These results are in disagreement with Jones et al. (2012), who revealed that biochar enhanced the activity of microbes in the second year of the study more than in the first and in the third years of study. In addition, the researchers claimed that application of biochar to soil only led to a little impact on the turnover of ^{14}C-labelled soil organic carbon and sugars. Also, Quilliam et al. (2012) claimed that there was no major change on microbial growth after three years of biochar application to soil. These findings are in agreement with the notion that adding biochar exerts an insignificant contribution to highly fertile temperate soils.

Table 3- Maximum rate, ^{14}C extent mineralisation, ^{14}C biomass uptake and ^{14}C activity remaining for arable sandy soil amended with and without fresh biochar (FB); and aged biochar (AB), over 300 days. Error bars are SEM (n=3)

Treatment	Day	Maximum rate (% h^{-1})	^{14}C extents mineralisation (%)	^{14}C biomass uptake (%) fixed k_{EC}	^{14}C activity remaining in soil (%)
Arable sandy	0	2.08 ± 0.04	44.40 ± 1.18	8.98 ± 3.64	46.61 ± 4.15
Control	60	2.89 ± 0.24	77.67 ± 11.19	27.95 ± 7.73	0.00 ± 0.00
	180	2.40 ± 0.15	66.92 ± 1.31	13.21 ± 3.45	19.87 ± 4.63
	300	1.64 ± 0.06	75.79 ± 10.41*	13.71 ± 2.54	10.50 ± 8.43
FB	0	2.18 ± 0.10	45.97 ± 3.66	9.20 ± 0.98	44.83 ± 3.72
	60	4.23 ± 1.03	87.26 ± 11.36	4.28 ± 1.51	0.00 ± 0.00
	180	3.37 ± 0.48	72.51 ± 6.20	6.94 ± 1.42	20.55 ± 6.25
	300	2.40 ± 0.51	73.60 ± 7.53*	16.41 ± 3.64	9.98 ± 10.23
AB	0	2.13 ± 0.41	42.17 ± 2.13	10.70 ± 1.16	47.13 ± 2.21
	60	3.42 ± 0.46	78.27 ± 7.43	27.29 ± 1.15	0.00 ± 0.00
	180	3.54 ± 0.55	73.83 ± 4.60	8.99 ± 1.63	17.17 ± 4.44
	300	3.23 ± 0.57	84.30 ± 5.12*	14.65 ± 1.80	1.04 ± 6.80

Values in asterisk indicate significance at P<0.05

The incorporation of ^{14}C-glucose into the microbial biomass also did not show any changes in the biomass. In addition, the mineralisation of ^{14}C-glucose in all treatments was constantly higher than the uptake of ^{14}C-glucose into the microbial biomass. Amending soil with fresh and aged biochar increased the amounts of both oxidizable and recalcitrant carbon in the soils, which would have influenced microbial activity. Therefore, the ^{14}C-glucose mineralisation increased, resulting in a decrease in ^{14}C-uptake. In contrast, positive priming effects of the activity of microbes in degraded/stressed soils following addition of peanut shell and sugarcane-bagasse-derived biochar was observed by Nie et al. (2018). The high sand content (66%) and lower CEC (9.24 meq 100 g^{-1}) of the arable sandy soil were unfavourable for the wood biochar to interact with to ensure any significant change in microbial activities.

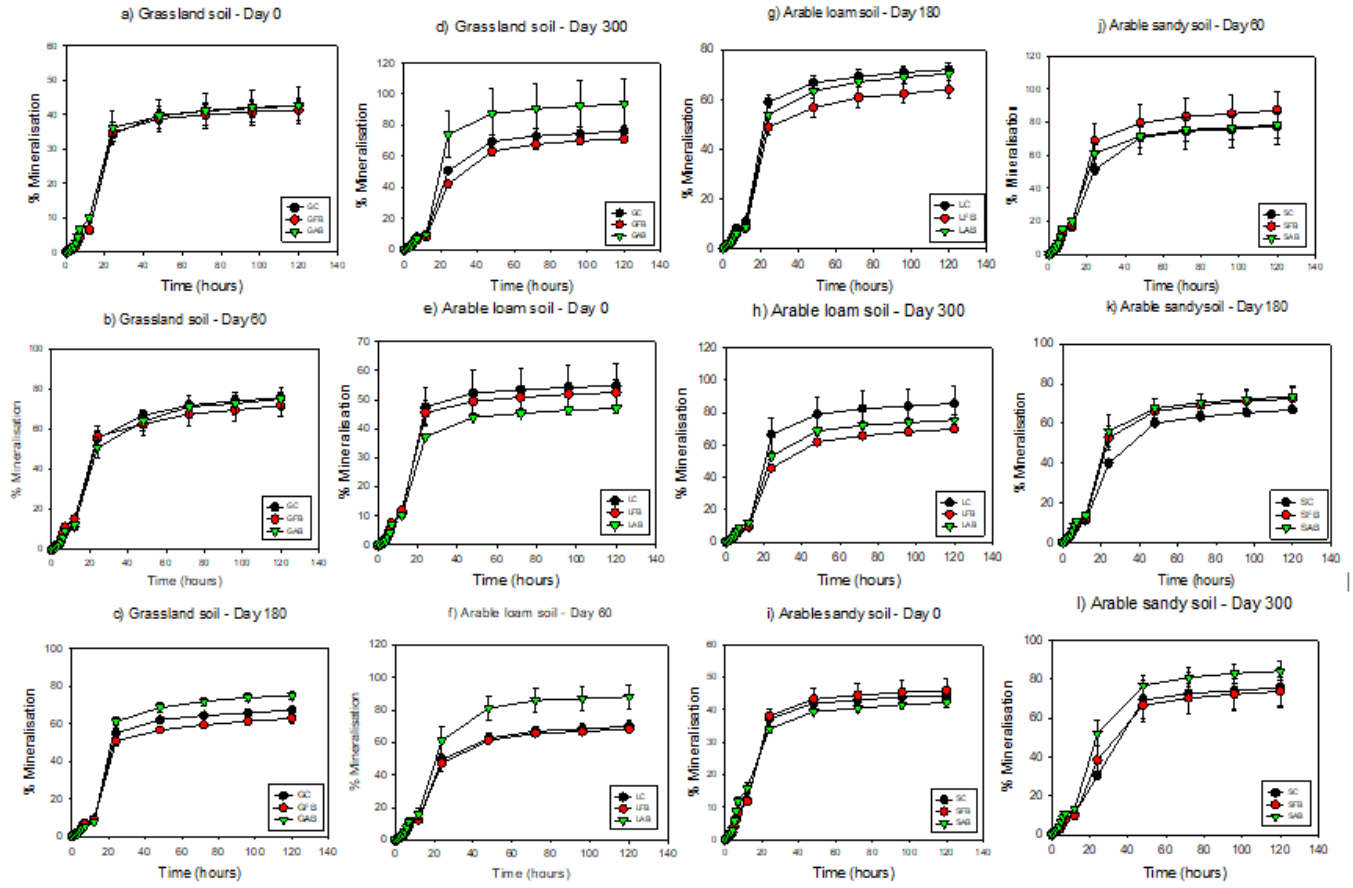

Figure 4- Mineralisation of ^{14}C-glucose of the a, b, c, d) grassland; e, f, g, h) arable loam and i, j, k, l) arable sandy soils on days 0, 60, 180 and 300, amended with fresh biochar (FB) and aged biochar (AB); and with no biochar. Error bars are SEM (n=3)

3.2.2. Experiment 2

The mineralisation of ^{14}C-glucose was low in the well-managed soil (fertilised soil) (Table 3 and Table 4) over a month. This result contrasted with the findings of experiment one, where the mineralisation of ^{14}C-glucose was higher. Moreover, the maximum rates of ^{14}C-glucose mineralisation did not show a constant pattern. Generally, with a higher application rate of biochar (5%) and finer particle size of biochar (1 and 0.1 mm) the highest and lowest maximum rates of mineralisation were observed on day 0 (1.20% h^{-1} and 0.47% h^{-1}) respectively ($P<0.05$), (Table 3). Nevertheless, the finer particle size (0.1 mm) significantly increased ($P<0.05$) mineralisation of ^{14}C-glucose at the higher dosage of biochar (5%) on the final day of incubation. In terms of the ^{14}C-glucose incorporation into the microbial biomass, the findings exhibit that at both dosages (2% and 5%), soil amended with biochar decreased the microbial biomass ($P<0.05$) in comparison with the un-amended soil (Tables 3 and 4).

Table 3- Maximum rates, ^{14}C extent mineralisation, ^{14}C biomass uptake and ^{14}C activity remaining for the fertilised soil (F) and unfertilised soil (UF) amended with 5% biochar and with no biochar (Control) over a month. Error bars are SEM (n=3). Values in asterisk indicate significance at P<0.05.

Treatment (mm)	*Day*	*Maximum rate (% h^{-1})*	*^{14}C extents mineralisation (%)*	*^{14}C biomass uptake (%)fixed k_{EC}*	*^{14}C activity remaining in soil (%)*
F Control	0	1.01 ± 0.10*	12.02 ± 1.23	93.22 ± 11.34	0.00 ± 0.00
	30	0.76 ± 0.12	10.34 ± 0.84	47.67 ± 5.45	37.78 ± 7.78
F 5% (2)	0	0.99 ± 0.08*	9.84 ± 0.47	23.54 ± 1.57*	62.91 ± 2.33
	30	0.97 ± 0.18	12.31 ± 1.15	29.04 ± 3.06*	53.50 ± 6.80
F 5% (1)	0	1.20 ± 0.19*	9.66 ± 0.18	27.17 ± 4.80*	59.80 ± 3.11
	30	0.86 ± 0.08	13.09 ± 1.13	41.19 ± 2.21*	40.33 ± 2.92
F 5% (0.5)	0	0.76 ± 0.03	8.08 ± 0.22	31.65 ± 7.49*	57.39 ± 8.45
	30	0.82 ± 0.12	13.32 ± 0.5	37.08 ± 4.50*	44.70 ± 7.56
F 5% (0.1)	0	0.47 ± 0.02	7.97 ± 0.94	30.05 ± 2.53*	58.47 ± 3.37
	30	0.85 ± 0.09	16.15 ± 0.52*	42.65 ± 2.05*	35.35 ± 7.59
UF Control	0	0.94 ± 0.15*	11.55 ± 0.39	41.20 ± 5.40	43.04 ± 7.20
	30	1.36 ± 0.08	19.09 ± 1.22	38.31 ± 4.13	35.12 ± 11.47
UF 5% (2)	0	0.88 ± 0.06	10.27 ± 1.15	6.05 ± 0.89*	79.21 ± 5.73
	30	2.12 ± 0.01	24.20 ± 0.17	26.80 ± 1.90*	40.78 ± 6.83
UF 5% (1)	0	0.74 ± 0.15	12.19 ± 0.51	9.15 ± 4.21*	74.13 ± 4.71
	30	1.95 ± 0.30	22.26 ± 1.01	31.28 ± 2.84*	38.12 ± 10.72
UF 5% (0.5)	0	0.63 ± 0.05	11.83 ± 1.49	4.37 ± 1.39*	78.49 ± 3.38
	30	1.86 ± 0.28	25.37 ± 1.26	31.59 ± 1.89*	33.44 ± 10.71
UF 5% (0.1)	0	0.36 ± 0.03	11.96 ± 0.09	8.17 ± 1.56*	75.79 ± 4.47
	30	1.51 ± 0.05	30.92 ± 0.73*	21.40 ± 1.97*	36.70 ± 8.71

Table 4- Maximum rates, ^{14}C extent mineralisation, ^{14}C biomass uptake and ^{14}C activity remaining for the fertilised soil (F) and unfertilised soil (UF) amended with 2% biochar and with no biochar (Control) over a month. Error bars are SEM (n=3). Values in asterisk indicate significance at P<0.05

Treatment (mm)	*Day*	*Maximum rate (% h^{-1})*	*^{14}C extents mineralisation (%)*	*^{14}C biomass uptake (%) fixed k_{EC}*	*^{14}C activity remaining in soil (%)*
F Control	0	1.01 ± 0.10*	12.02 ± 1.23	93.22 ± 11.34	0.00 ± 0.00
	30	0.76 ± 0.12	10.34 ± 0.84	47.67 ± 5.45	37.78 ± 7.78
F 2% (2)	0	1.05 ± 0.32	11.30 ± 1.18	72.25 ± 6.55*	11.61 ± 11.60
	30	1.10 ± 0.07	12.89 ± 0.96	61.61 ± 12.30	20.33 ± 15.47
F 2% (1)	0	0.82 ± 0.16	9.80 ± 0.40	73.49 ± 11.18*	13.08 ± 13.58
	30	0.73 ± 0.03	12.78 ± 0.64	39.63 ± 2.23*	42.75 ± 6.79
F 2% (0.5)	0	1.19 ± 0.11	9.66 ± 0.22	45.65 ± 0.86*	41.28 ± 3.39
	30	1.07 ± 0.27	13.77 ± 1.72	46.90 ± 6.51*	33.17 ± 11.27
F 2% (0.1)	0	1.07 ± 0.13	9.68 ± 0.24	48.92 ± 4.70*	37.96 ± 7.72
	30	1.02 ± 0.10	13.79 ± 1.41	43.48 ± 7.70*	36.84 ± 8.81
UF Control	0	0.94 ± 0.15*	11.55 ± 0.39	41.20 ± 5.40	43.04 ± 7.20
	30	1.36 ± 0.08	19.09 ± 1.22	38.31 ± 4.13	35.12 ± 11.47
UF 2% (2)	0	0.79 ± 0.07	10.87 ± 0.59	11.62 ± 2.88*	73.35 ± 2.26
	30	1.26 ± 0.17	22.26 ± 2.06	30.31 ± 3.85*	38.14 ± 7.82
UF 2% (1)	0	0.81 ± 0.09	11.65 ± 1.12	13.40 ± 0.97*	70.04 ± 5.82
	30	1.31 ± 0.20	26.04 ± 3.44	24.12 ± 5.61*	38.03 ± 17.72
UF 2% (0.5)	0	0.56 ± 0.05	13.39 ± 0.59	14.45 ± 0.27*	67.15 ± 5.44
	30	1.43 ± 0.17	23.43 ± 1.49	36.02 ± 2.44*	31.37 ± 11.68
UF 2% (0.1)	0	0.95 ± 0.01	11.98 ± 1.16	13.67 ± 1.43*	69.30 ± 4.36
	30	1.86 ± 0.35	29.75 ± 2.80	23.34 ± 3.45*	34.44 ± 15.64

In comparison to the mineralisation of ^{14}C-glucose the unmanaged soil (unfertilised soil) displayed the same pattern as the well-managed soil (fertilised soil) (Tables 3 and 4). The mineralisation of ^{14}C-glucose was relatively lower than the incorporation of ^{14}C-glucose into the biomass. The smallest particle size (0.1 mm) increased the ^{14}C-glucose mineralisation ($P<0.05$) after a month (30 days) contact time at a higher dosage (5%) (Table 3). Meanwhile, the maximum rates of mineralisation constantly increased over the course of the study. For instance, on days 0 and 30 the maximum rate of ^{14}C-glucose mineralisation ranged from 0.36 to 0.95 (% h^{-1}) and 1.26 to 2.12 (% h^{-1}), respectively (Tables 3 and 4). In addition, the maximum rate in the untreated soil on day 0 (Table 4) was significantly higher ($P<0.05$) than at 5% dosage, as well as at 0.1 mm of particle size (Table 3). Also, the biomass uptake of ^{14}C-glucose had a similar pattern as fertilised soil where the value of the biomass uptake decreased at the higher application rate (5%) of biochar compared to the un-amended soil ($P<0.05$). Mineralisation and biomass uptake of ^{14}C-glucose in both soils (fertilised and unfertilised soils) did not significantly affect lower rate of biochar application (2%), as well as various particle sizes of biochar (Table 4). This finding is in agreement with Jones et al. (2012) and Quilliam et al. (2012). Though, at 5% application rate of biochar, the extent of mineralisation of ^{14}C-glucose increased over the period of the study and also in both well-managed and unmanaged soils ($P<0.05$). These results indicate that the effect of amending soil with biochar exhibited an increase in microbial activity at higher application rates. Likewise, the improvement in the mineralisation of ^{14}C-carbon proves that there was a positive priming and degradation of the labile carbon fractions of biochar in soil (Hamer et al. 2004). Furthermore, Quilliam et al. (2012) observed that there was a major change in the quality of soil and microbial biomass in the treatments with higher application rates of biochar (25+25t ha^{-1} and 50+50t ha^{-1}) in contrast to the treatments with lower rates of biochar application (25t ha^{-1} and 50t ha^{-1}) after a longer period of biochar application (more than 3 years). The authors stated that soil nutrient status and soil structure improved, making it suitable for microbial habitation when higher dosages of biochar were applied.

In addition, our findings reveal that smaller particle sizes stimulated more ^{14}C-glucose mineralization than the greater sizes in fertilised and unfertilised soils. The results are in agreement with Sigua et al. (2014), where, finer particle size of biochar (<0.42 mm) increased the mineralisation rate and amount of CO_2 evolution compared to the larger particle size of biochar (>2 mm) due to higher surface area of the former. Similarly, finer-particle, spent mushroom-derived biochar (<0.5 mm) produced at 700 ^{o}C accelerated the release of phosphates and improved bacterial species richness in soils, compared to larger particle sizes (>0.5 mm) (Sarfraz et al. 2020). In support, Zhao et al. (2020) further attributed enhanced microbial activity and biomass by finer-particle (<1 mm) wood biochar (450 ^{o}C) to higher N nutrient release and degradation. Generally, uptake of ^{14}C-glucose in the control soils (fertilised and unfertilised) of this study were much higher than in soils amended with biochar. However, fertilisation increased the ^{14}C-glucose uptake by 50%, indicating the necessity of nutrient availability for glucose uptake and carbon turnover by microorganisms (Table 3) (Liang et al. 2019). The availability of nutrients and glucose in soils thus encourages microbial cellular development and carbon sequestration, but as time increases, the uptake rate decreases markedly. In fertilised soils, increases in biochar concentration significantly ($P<0.05$) reduced ^{14}C-glucose uptake and it was observed that at 2% concentration, the finer-particle biochars (0.1 and 0.5 mm) further reduced ^{14}C-glucose uptake significantly compared to larger particle sized biochars (1 and 2 mm). It seems that larger surface area and glucose adsorption sites of the finer particles inhibited glucose availability for microbial uptake and utilization.

On the contrary, lower nutrient content in unmanaged (fertilised) soil resulted in a reduction in microbial biomass uptake and a higher mineralisation rate of ^{14}C-carbon in comparison to the well-managed (fertilised) soil (Table 3). The scarce source of nutrients in unmanaged soil limits microbial ^{14}C-glucose uptake and further growth of microbes in the soils. This interpretation is supported by Zhang et al. (2014), in which the authors demonstrated that inadequate nutrients and a lack of available carbon in a larger-textured soil make the soil unsuitable for microbial growth. As biochar concentration increased, there was also a corresponding decrease in ^{14}C-glucose biomass uptake, but as incubation time increased to 30 days, ^{14}C-glucose uptake increased remarkably in the unfertilised soils. This shows that despite adsorption of glucose in soils, the extent of adsorption is reversible and in support of biochar oxidation, carbon sequestration and turnover rate regulation over time.

3.2.3. Effect of biochar on ammonium and nitrate ion loss through leaching in the arable loam, arable sandy and grassland soils (experiment 1).

The amount of ammonium ions leached increased consistently in all of the soils over the course of the study ($P<0.05$) (Figures 5a, b and c). However, the concentration of ammonium ions in all soils treated with fresh and aged biochar significantly decreased compared to untreated soils. Furthermore, in the first leaching experiment there was no change in the concentration of ammonium ions between the treatments with biochar and with no biochar ($P>0.05$). But, in the final leaching experiment the biochar treatment decreased ammonium leaching significantly ($P<0.05$) from 0.34 mgL^{-1} (control) to 0.06 mg/L (fresh biochar) and 0.14 mg/L (aged biochar) (Figure 5a); and the amount of ammonium leachate in the grassland soil peaked. In addition, the concentration of ammonium ions in the arable loam soil decreased from 0.25 to 0.09 mg/L in fresh biochar treatment and 0.15 mg/L in aged biochar treatment (Figure 5b). Meanwhile, the arable sandy soil ammonium leaching also reduced significantly ($P<0.05$) from 0.88 to 0.27 mg/L in aged biochar treatment and 0.13 mg/L in fresh biochar treatment (Figure 5c). Yao et al. (2012), demonstrated that nine various types of biochar can adsorb ammonium ions ranging from 1.8% to 15.7% owing to the high cation exchange capacity of biochar.

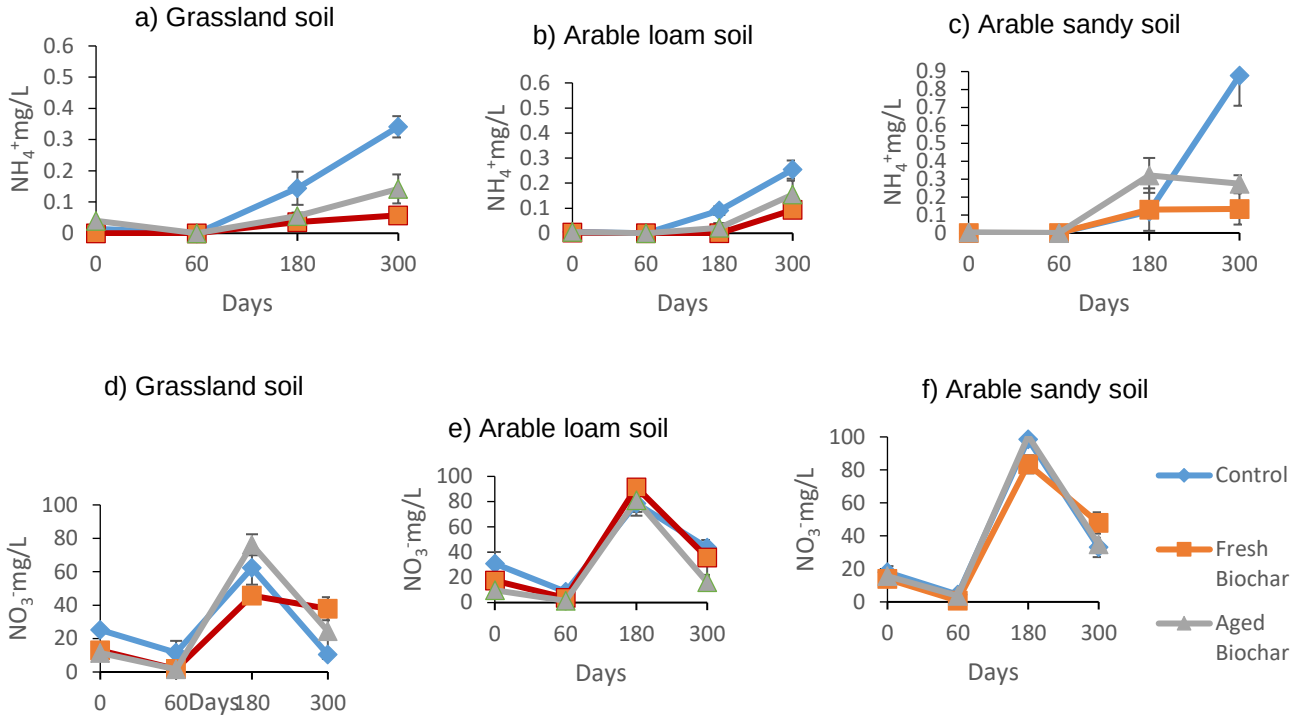

Figure 5- Concentration of ammonium (NH_4^+) and nitrate (NO_3^-) ions in the leachate of the a) and d) grassland, b) and e) arable loam and c) and f) arable sandy soils treated with fresh and aged biochar; and with no biochar, over 300 days. Error bars are SEM (n=3)

Nitrate ion concentration in the soil leachates fluctuated over the course of the study. The trend of nitrate leaching in biochar treatments did not display a consistent pattern and the differences were also insignificant (P>0.05). For example, in the first leaching experiment the concentration of nitrate ions was low. Then it increased in the middle of the leaching event and then decreased at the end of the leaching experiment (Figures 5d, e and f). This indicates that the nitrification process and reversible sorption happened, whereas the inhibition of nitrification took place at the end of the leaching experiment. Nevertheless, the mechanisms behind this process are unclear. Moreover, biochar treatments in the arable sandy soil did not show any effect, though biochar treatments were observed only to decrease the concentration of nitrate ions leached on days 0 and 60 in the arable loam and grassland soils (Figures 5d, e and f).

3.2.4. Effect of biochar on ammonium and nitrate ion loss through leaching in fertilised and unfertilised soils (experiment 2)

The amount of ammonium ions leached from both well-managed (fertilised) soil and unmanaged (unfertilised) soil was very small; and ranged from 0.00 to 0.07 mg/L. In the beginning of the leaching process, the concentration of ammonium ions in the untreated fertilised soil was higher compared to the soils with biochar treatments. However, the leaching of ammonium ions was observed to be really low and the differences were insignificant in the final leaching event which was on day 30 (Table 5). In addition, the concentration was too low to be observed in this study. However, the amount of nitrate ions leached was higher than that of ammonium ions in both soils (Table 5). Results show that biochar treatments affected nitrate leaching only in the early stage of a leaching event. For instance, the concentration of nitrate ions slightly increased at the higher application rate of biochar (5%) with 0.1 mm particle size on day 0, though at the final leaching event (day 30) the biochar did not show any effect (Table 5). An increase in nitrate leaching is not uncommon for biochar-amended soils. Since ammonium ion concentration was significantly low, we rule out nitrification occurrence. Rather it is partly due to vertical transport of smaller particles of biochar upon moisture addition (Wang et al. 2013) and electrostatic repulsion due to more negative charges of O-containing functional groups on biochar surfaces (Zhang et al. 2020). This would have been more obvious in the finer-particle-sized and high-dosage of biochar. Nevertheless, during the final leaching process, unmanaged soil (unfertilised) treated with (2, 1, 0.5 and 0.1 mm) biochar reduced the concentration of nitrate ions in the leachate at both higher and lower application rates in comparison to the control treatment (Table 5). But in fertilised soils, 2, 1 and 0.5 mm biochars (5%) either increased or had no effect on leachate formation after 30 days of incubation. Obviously, this shows reversible nitrate adsorption capacities of the biochars and microbial influence to enhance availability of the nitrates following increase in contact time.

Table 5- Effect of ammonium (NH_4^+) and nitrate (NO_3^-) leaching, in fertilised and unfertilised soils treated with 2% and 5% biochar; and with no biochar, over a month. Error bars are SEM (n=2). Values in asterisk indicate significance at P<0.05

Variable	*Treatments (mm)*	*Fertilised Day 0 (mg/L)*	*Fertilised Day 30 (mg/L)*	*Unfertilised Day 0 (mg/L)*	*Unfertilised Day 30 (mg/L)*
Ammonium (NH_4^+) Leaching	Control	0.07 ± 0.05	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
	2% (2)	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.00	0.01 ± 0.01
	2% (1)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00*	0.00 ± 0.00
	2% (0.5)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	2% (0.1)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00*	0.00 ± 0.00
	5% (2)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
	5% (1)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	5% (0.5)	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
	5% (0.1)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Nitrate (NO_3^-) Leaching	Control	19.70 ± 0.45	15.92 ± 2.42	11.48 ± 0.63	15.04 ± 0.92
	2% (2)	16.60 ± 3.50	15.81 ± 2.54	12.08 ± 0.88	7.04 ± 4.58
	2% (1)	16.28 ± 0.33	9.72 ± 4.36	10.45 ± 0.30	9.38 ± 0.11
	2% (0.5)	17.70 ± 0.55	5.29 ± 2.87	10.48 ± 0.63	12.11 ± 2.93
	2% (0.1)	18.83 ± 0.88	4.47 ± 0.76	10.90 ± 0.05	8.03 ± 0.54
	5% (2)	17.53 ± 2.63	17.11 ± 6.01	10.83 ± 0.08	4.88 ± 0.20*
	5% (1)	11.55 ± 1.60	22.08 ± 3.64	9.28 ± 0.43	3.66 ± 0.62*
	5% (0.5)	15.30 ± 1.15	14.15 ± 2.34	9.63 ± 0.73	2.18 ± 0.12*
	5% (0.1)	22.30 ± 1.75*	6.00 ± 3.47	12.53 ± 0.13*	5.25 ± 0.37*

4. Conclusions

Pyrolysis of biomass to produce biochar sustainably stabilizes carbon and can enhance soil and agronomic properties upon application. This study revealed that smaller particle sizes and higher application rate of biochar mineralised more ^{14}C-carbon than greater sizes. Soils with poor nutrient contents have more advantages than soils which are rich in nutrients with respect to the microbial growth. These benefits were further enhanced when higher application rates were applied. Finer particle sizes of biochar were also more beneficial. In addition, biochar had a higher preference to adsorb ammonium ions rather than nitrate ions. This can minimise the loss of ammonium ions through leaching and reduces the potential for eutrophication. The soils investigated in this study are temperate soils. This study provides vital information with regards to the use of biochar in the temperate region to sequester carbon, ameliorate acidic soils and manage ammonium ion inputs. The effects of biochar are dependent on various factors, such as the kind of soils used, the dosage, as well as the particle size of biochar. Consequently, the results of this study are useful for understanding the factors that influence the application of biochar in agricultural fields. These factors need to be further assessed prior to the application of biochar on a broader and wider scale.

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