

Stability and stabilisation of biochar and green manure in soil with different organic carbon contents

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Abstract. Due to its recalcitrance against microbial degradation, biochar is very stable in soil compared to other organic matter additions, making its application to soils a suitable approach for the build-up of soil organic carbon (SOC). The net effects of such biochar addition also depend on its interactions with existing organic matter in soils. A study was established to investigate how the status of pre-existing soil organic matter influences biochar stabilisation in soil in comparison to labile organic additions. Carbon loss was greater in the C-rich sites (C content 58.0 g C/kg) than C-poor soils (C content 21.0–24.0 g C/kg), regardless of the quality of the applied organic resource. Biochar-applied, C-rich soil showed greater C losses, by >0.5 kg/m².year, than biochar-applied C-poor soil, whereas the difference was only 0.1 kg/m².year with *Tithonia diversifolia* green manure. Biochar application reduced the rate of CO₂-C loss by 27%, and *T. diversifolia* increased CO₂-C losses by 22% in the C-poor soils. With biochar application, a greater proportion of C (6.8 times) was found in the intra-aggregate fraction per unit C respired than with green manure, indicating a more efficient stabilisation in addition to the chemical recalcitrance of biochar. In SOC-poor soils, biochar application enriched aromatic-C, carboxyl-C, and traces of ketones and esters mainly in unprotected organic matter and within aggregates, as determined by Fourier-transform infrared spectroscopy. In contrast, additions of *T. diversifolia* biomass enriched conjugated carbonyl-C such as ketones and quinones, as well as CH deformations of aliphatic-C mainly in the intra-aggregate fraction. The data indicate that not only the stability but also the stabilisation of biochar exceeds that of a labile organic matter addition such as green manure.

Additional keywords: biochar, SOC stabilisation, SOM degradation, stability.

Introduction

Biochar is highly recalcitrant to decomposition (Schmidt and Noack 2000), typically has a high ratio of carbon (C) to nitrogen (N), and has the ability to increase soil cation exchange capacity (CEC) (Tryon 1948; Mikan and Abrams 1995; Topoliantz *et al.* 2002; Lehmann *et al.* 2006; Liang *et al.* 2006). Due to its long half-life (Baldock and Smernik 2002), biochar could be one of the best approaches to sequester C in soils, contributing to a net carbon dioxide withdrawal from the atmosphere (Lehmann 2007; Laird *et al.* 2009). In addition to improving nutrient retention ability (Glaser *et al.* 2002; Bélanger *et al.* 2004; Major *et al.* 2009), water-holding capacity (Glaser *et al.* 2002), and microbial functions of soils (Thies and Rillig 2009), substantial increases in soil organic carbon (SOC) have been shown with the application of biochar in highly SOC-depleted soils (Kimetu *et al.* 2008; Novak *et al.* 2010). Biochar can thus be a valuable tool in enhancing fertility and C stocks in soils that are highly depleted of SOC.

However, the ability of any soil to stabilise both native and added C varies to a great extent depending on the chemical and physical properties of the mineral matrix as well as the morphology and the chemical structure of the pre-existing soil organic matter (SOM) (Baldock and Skjemstad 2000). In the recently developed C saturation concept, Six *et al.* (2002),

Stewart *et al.* (2007, 2008), and Gulde *et al.* (2008) indicate that the physicochemical characteristics of soils put a ceiling on the protective capacity of SOC pools by soil aggregates and clay minerals, which limits increases in SOC even with the addition of organic inputs (e.g. Campbell *et al.* 1991; Paustian *et al.* 1997; Solberg *et al.* 1997; Kimetu *et al.* 2008). Six *et al.* (2002) and Stewart *et al.* (2007) suggested that soils with high OC status have a lower C stabilisation efficiency than soils of low OC status. We therefore hypothesise that stabilisation of biochar C will be higher in soils containing low amounts of OC than in those containing high amounts of OC. While we understand that there are complex interactions between the quality of added C and both the quality and quantity of pre-existing SOM, we cannot yet predict the dynamics of stabilisation for biochar added to a given soil.

These relationships may be further complicated by the effect that biochar may have on existing SOM. Wardle *et al.* (2008) showed greater losses of an organic horizon after the addition of char; however, other authors did, in some cases, observe a lower C emission after biochar addition to soil (Kuz'yakov *et al.* 2009; Spokas *et al.* 2009; Liang *et al.* 2010). Therefore, the main objective of this study was to investigate how status of pre-existing SOM influences biochar stabilisation in soil in comparison to labile organic additions from green manure.

without the litter layer was measured gravimetrically through 3 growing seasons using the soda lime technique (Edwards 1982; Grogan 1998), once per month after a bi-weekly measurement during the first 2 months to capture the initial mineralisation after site preparation (total of 30 flux measurements per treatment per farm). Measurements were done in static vented chambers (PVC pipes with an internal area of 0.0314 m²) placed on top of the soil with permanent rims inserted to a depth of 1 m, using an aliquot of 10 g soda lime as the CO₂ trap (modified from Puget and Drinkwater 2001). Two flux measurements were taken per plot with 3 plots (1 plot each installed on 3 different farms) for each treatment (control, *T. diversifolia*, and biochar on each 5- and 105-year-old conversion, in comparison to forest plots). Flux measurements were also done in 6–7 randomly selected spots on the untreated farm plots, which revealed minimal spatial variability within a given farm.

Soda lime traps were weighed using a Mettler Toledo weighing scale with an accuracy of up to 1 mg. Weighing was done the day before deployment after traps were pre-dried at 100°C for 24 h. They were immediately sealed in airtight, wide-mouth vials and transported for deployment in the field within 1 h after the pre-drying process. After exposure for 24 h inside the closed chamber in the field, the trap was retrieved and sealed immediately, then transported instantaneously to the laboratory for post-drying (at 100°C for 24 h) and final weighing. The amount of CO₂ absorbed by the soda lime trap was determined by mass difference between initial weight of the trap and the final weight. Appropriate blank treatments without soil were used to correct for CO₂ absorption during handling (Major *et al.* 2010). To establish the effect of different treatments on C mineralisation obtained by the soda-lime technique, net CO₂-C loss was calculated by subtracting CO₂-C loss in control plots from CO₂-C loss in plots that received either biochar or *T. diversifolia*.

Soil sampling and analyses

To investigate the influence of the added OM on entire SOC, soil was sampled using a soil auger (diameter 55 mm) in all plots from the plough layer (determined to be 0–0.1 m) after 3 cropping seasons (24, 13, and 7 months after the first, second, and third seasons' application, respectively). Pooled samples from 6–7 randomly selected sampling points in each plot were thoroughly mixed in a bucket, from which a subsample was drawn, dried, and ground to pass through a 2-mm sieve. A smaller subsample of ~10 g was ground further into fine powder for total C and δ¹³C analyses. A repeated measurement was done on the same sample, yielding similar results (<10% coefficient of variation), and hence a mean of the duplicate measurements is reported. This procedure led to 3 replicate measurements (equalling to 3 farms) for each conversion age. Microbial biomass was measured on the same samples taken in September 2006, using fumigation extraction (Brookes *et al.* 1985).

Soil fractionation

To study C stabilisation, pooled soil samples from across 3 replicate farms from each of the selected representative conversion ages (5, 20, and 105 years) were used. A

subsample of 15 g was taken from a main sample from each of the 3 replicate farms, pooled together, mixed thoroughly, and then fractionated by density separation using sodium iodide (1.8 g/cm³) into free light fraction, intra-aggregate light fraction, and organo-mineral complexes following the method of Sohi *et al.* (2001). The various soil fractions were analysed for total C and δ¹³C. Samples were analysed for organic C contents with a Europa ANCA-GSL CN analyser (PDZ Europa Ltd, Sandbach, UK). Natural abundance of ¹³C for the same sample was determined on a coupled Europa 20–20 continuous flow isotope ratio mass spectrometer (PDZ Europa Ltd, Sandbach, UK) following combustion at 1000°C. The δ¹³C values were expressed relative to Vienna-Pee Dee Belemnite (V-PDB) standard.

Fourier-transform infrared analyses

In addition, spectroscopic analyses were done on the fractions to test whether the quality of OM additions affects the quality of stabilised SOM. These investigations were done by Fourier-transform infrared (FTIR) analyses. Ground OM (0.3–0.5 mg) was embedded in potassium bromide (KBr) pellets (99.5–99.7 mg) and measured on a Mattson mode 5020 FTIR spectrometer with a 4 cm⁻¹ resolution and 100 scans between wavenumbers of 4000 and 400 cm⁻¹ (Chun *et al.* 2004). To analyse C forms from the FTIR spectra, we subtracted the background of the KBr window, automatically corrected the baseline and smoothed the spectra, identified the peaks, and normalised the spectra on a reduced portion of the wavenumbers (4000–800 cm⁻¹) using OMNIC version 7.2 (Thermo Electron Corp., Woburn, MA).

Calculations of the carbon source

The isotopic differences between the added OM that is exclusively derived from C₃ plants, and the SOC in old conversions which were continuously cropped with maize for >100 years (C₄ plants), served as a tool to partition between added and native C (Bernoux *et al.* 1998; Spaccini *et al.* 2002). The carbon isotopic composition of SOC is comparable to that of the source plant material, even though small changes may occur due to isotope discrimination (Bernoux *et al.* 1998). Consequently, the natural abundance of ¹³C, measured by mass spectrometry, can be used to partition C into SOC from the different organic amendments using the mass balance equation shown below (Balesdent and Mariotti 1996; Huggins *et al.* 1998, 2007; Solomon *et al.* 2002). This approach could only reliably be interpreted in older conversions, since SOC in younger conversions was dominated by C₃-derived forest SOC, and the C isotopic composition does not differ between the forest SOC and the applied OC:

$$\begin{aligned} \%C \text{ derived from added OC} = & \\ & \frac{[(\delta^{13}\text{C Soil}_{\text{final}} - \delta^{13}\text{C Soil}_{\text{initial}})]}{(\delta^{13}\text{C Added OC} - \delta^{13}\text{C Soil}_{\text{initial}})} * 100 \end{aligned}$$

Forest sites were included as a reference for C sequestration potential.

Statistical analyses

The statistical differences between experimental treatments and ecosystem degradation stages were determined through Analysis of Variance (General ANOVA procedure) in GENSTAT version 8.2 (Rothamsted Experimental Station 2005). Mean separation was computed using least significant difference at $P=0.05$. For CO₂-C loss and C in fractions, differences between treatments were determined by regression analysis whereby the difference between slopes of any pair of curve fits was hypothesised to be unequal to zero.

Results

Soil organic carbon

Delta ¹³C ($\delta^{13}\text{C}$) shifted by -1.1 to -2.3‰ towards the C₃ signature with the application of either biochar or *T. diversifolia* biomass in the C-poor soils, with a slight shift (-0.4 to -0.8‰) in the moderately C-rich soils indicating C₃ enrichment but no significant impact on $\delta^{13}\text{C}$ signature in the C-rich soils (Table 3). Consequently, in the C-poor soils, an estimated 84% of the C applied with biochar was found in the top 0.1 m of the soil at the end of the 3 seasons, while in the *T. diversifolia* plots only ~24% of the C applied with *T. diversifolia* was found in the soil (Table 3). The recoveries in the C-rich and moderately C-rich soils are not considered further as the differences in isotope values were insufficient.

Carbon dioxide evolution

Soil C loss as gaseous CO₂ at the C-poor sites significantly ($P=0.0001$) differed as a result of applying different organic

amendments, with no significant ($P=0.903$) seasonal differences. Determined using the soda lime technique, cumulative CO₂-C loss in the C-poor sites was $\sim 4.1(\pm 0.6 \text{ s.e.}) \text{ g/m}^2\cdot\text{day}$ ($\sim 1500 \text{ g/m}^2\cdot\text{year}$) averaged over 444 days with the application of *T. diversifolia* leaf biomass, while C loss in biochar-applied plots was only $2.4(\pm 0.2) \text{ g/m}^2\cdot\text{day}$ ($\sim 880 \text{ g/m}^2\cdot\text{year}$) averaged over the same time period (Fig. 1a).

Carbon loss from control plots was $\sim 3.4(\pm 0.2) \text{ g/m}^2\cdot\text{day}$ ($1200 \text{ g/m}^2\cdot\text{year}$), which was $>300 \text{ g/m}^2\cdot\text{year}$ more than that from plots that received biochar and $\sim 270 \text{ g/m}^2\cdot\text{year}$ less than *T. diversifolia* plots (Table 3). Application of biochar thus reduced the rate of CO₂-C loss by $\sim 27\%$, while *T. diversifolia* increased C loss by 22% compared with the unamended control at the C-poor sites. Cumulative C loss from the forest floor in the C-poor soils was $2.8(\pm 0.4) \text{ g/m}^2\cdot\text{day}$ ($1000 \text{ g/m}^2\cdot\text{year}$) (17% higher than the C loss in biochar-applied plots but 32% and 18% lower than *T. diversifolia*-applied and control plots, respectively).

Although the application of *T. diversifolia* leaf biomass resulted in the highest increase in CO₂-C loss at the C-rich sites, the application of biochar and *T. diversifolia* did not differ significantly ($P>0.05$) from each other or from the unamended control (Fig. 1b). Throughout the entire measurement period, the forest floor maintained the lowest CO₂-C loss in comparison to the C-rich sites. Carbon loss at the C-rich sites was greater than at the C-poor sites regardless of the quality of the applied organic resource. For example, biochar applied to C-rich, less-degraded soil showed $>500 \text{ g C/m}^2\cdot\text{year}$ greater losses than on C-poor soil, while C-rich plots to which *T. diversifolia* was applied lost $\sim 100 \text{ g/m}^2\cdot\text{year}$ more than C-poor, highly degraded plots.

Table 3. Soil carbon changes with organic inputs of differing quality after 2 years of repeated application

Maize residue data are adapted from Kimetu *et al.* (2008) and inserted for comparison. Within columns, values followed by the same letter are not significantly ($P=0.05$) different from each other. Comparisons are done separately for each period of cultivation and for each column. n.d., No data; n.c., not calculated as isotope difference between soils was insufficient; recovery was calculated using isotopes, and non-recovered C includes mineralisation as well as physical loss (Nguyen *et al.* 2008); $n=3$

Treatment	Crop residues (t/ha)	Soil carbon content (g/kg)	$\delta^{13}\text{C}$ (‰)	Recovery (% added C found in soil)	Net CO ₂ -C loss (mg/m ² /day)
<i>105 years of continuous cultivation</i>					
Biochar	8.8b	30.5a	-20.6a	83.8a	-950
<i>T. diversifolia</i>	13.8a	26.8b	-20.4a	23.7b	741
Control	9.2b	21.1c	-18.3b	-	-
I.s.d. ($P=0.05$)	2.6	1.6	1.3	21.6	-
<i>35 years of continuous cultivation</i>					
Biochar	6.9b	32.6a	-20.0a	n.c.	n.d.
<i>T. diversifolia</i>	11.8a	30.1a	-20.3a	n.c.	n.d.
Control	4.9b	24.2a	-18.9b	-	-
I.s.d. ($P=0.05$)	3.0	9.8	0.9	-	-
<i>20 years of continuous cultivation</i>					
Biochar	6.6b	36.4a	-22.0a	n.c.	n.d.
<i>T. diversifolia</i>	12.7a	32.6a	-21.6b	n.c.	n.d.
Control	6.7b	32.6a	-21.2c	-	-
I.s.d. ($P=0.05$)	2.8	5.7	0.3	-	-
<i>5 years of continuous cultivation</i>					
Biochar	8.2a	65.2a	-24.6a	n.c.	187
<i>T. diversifolia</i>	10.5a	60.2a	-24.5a	n.c.	658
Control	10.7a	59.9a	-24.0a	-	-
I.s.d. ($P=0.05$)	2.8	6.4	1.2	-	-

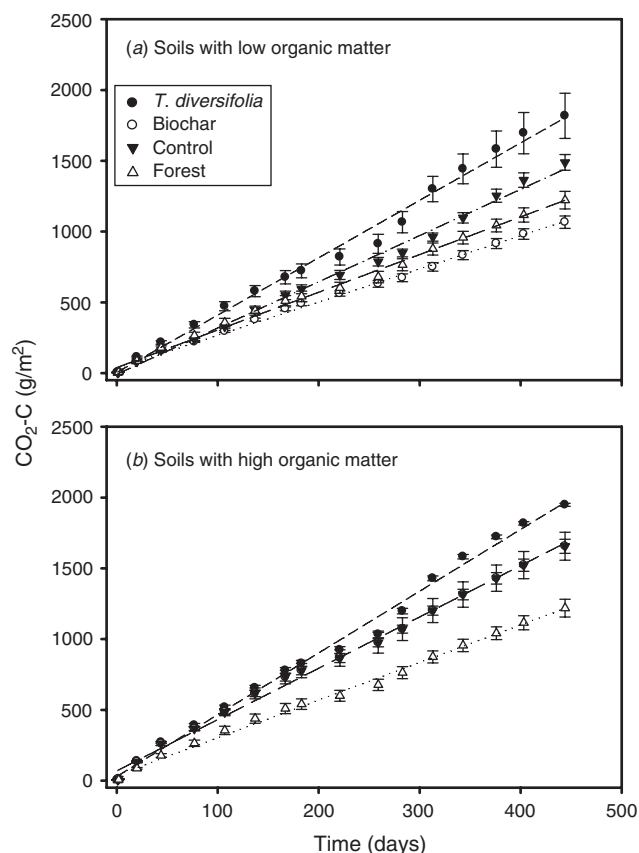


Fig. 1. Cumulative CO₂-C loss in (a) C-poor and (b) C-rich Ultisols under different management strategies, 2005–2006. Difference between treatment means was determined by regression analysis ($P=0.0001$); model: standard linear curve: $y=y_0+ax$. Error bars = s.e. ($n=3$).

Organic carbon in physical fractions

Effects of added organic matter on C contents were significant only in the free light and intra-aggregate fractions ($P<0.0001$), but not in the organo-mineral fraction ($P=0.347$) (Fig. 2). On average over the entire degradation sequence, significantly ($P=0.042$) more C entered free light and intra-aggregate fractions when biochar was applied than after application of *T. diversifolia* mulch. In both fractions, the difference between biochar and *T. diversifolia* additions significantly ($P<0.1$) increased from C-rich to C-poor soils (Fig. 2).

FTIR analyses of soils after organic matter additions

The structural composition of organic C changed with the application of biochar or *T. diversifolia* (Fig. 3). In C-poor soils, biochar application enriched the proportion of C=C of aromatic C (at 1558–1586 cm⁻¹), C=O stretching mainly of carboxylic C and traces of ketones and esters (at 1616.5 and 1697.8 cm⁻¹), and C–O stretching and OH deformations of carboxylic C (at 1385.2 cm⁻¹) mainly in the free light and intra-aggregate fractions (Fig. 3). In contrast, application of *T. diversifolia* biomass enriched conjugated carbonyl C (C=O) such as ketones and quinones (at 1623.6–1635.9 cm⁻¹) as well as CH deformations of aliphatic C (at 1384.5 cm⁻¹),

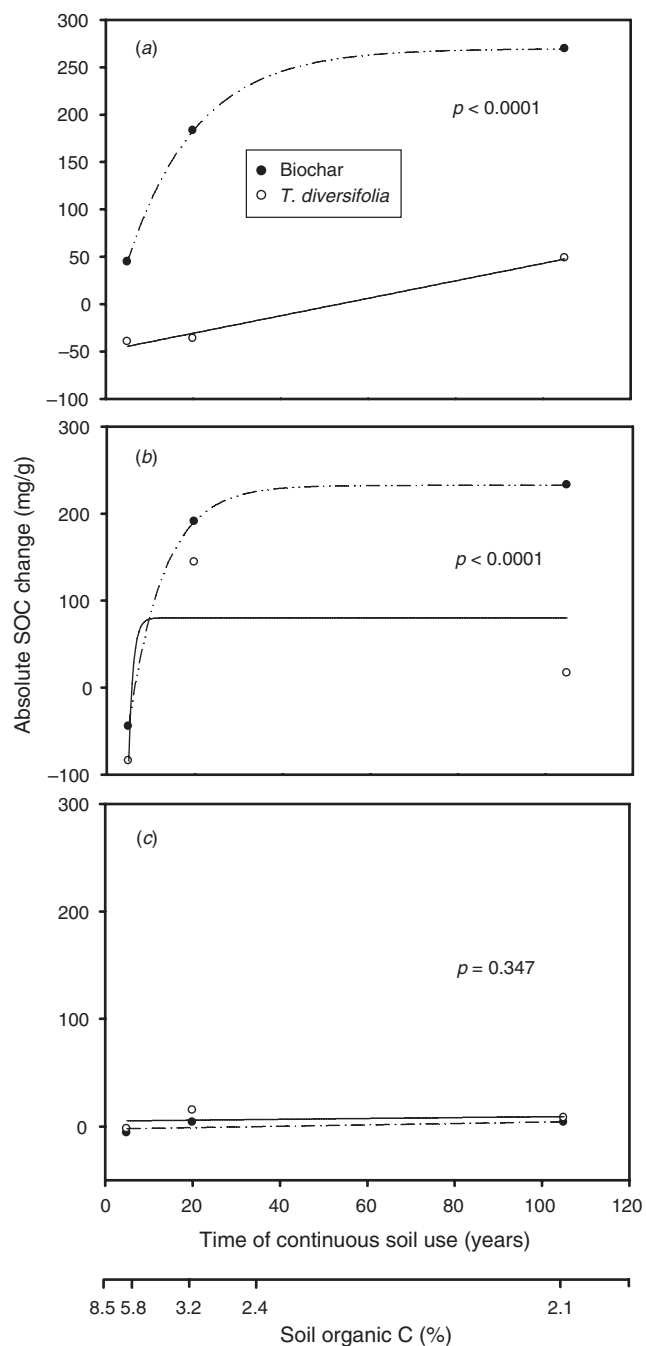


Fig. 2. Influence of organic matter additions on the quantity of SOC in different soil fractions: (a) free light fraction, (b) intra-aggregate fraction, (c) organo-mineral fraction. Measured samples were pooled across 3 replicate farms in each conversion age ($n=1$). Difference between the slopes was determined at $P=0.05$; models: 3-parameter double-exponential rise: $y=y_0+a[1-\exp(-bx)]$ or standard linear curve: $y=y_0+ax$.

mainly in the intra-aggregate fraction. The broad intense bands at ~3443 and 3421 cm⁻¹ representing OH stretching vibrations of H-bonded hydroxyl (O–H) groups of phenol with traces of amine stretches (N–H) predominated in the free light and intra-aggregate fractions of soils that received either biochar or *T. diversifolia*, while in the control soils this band is

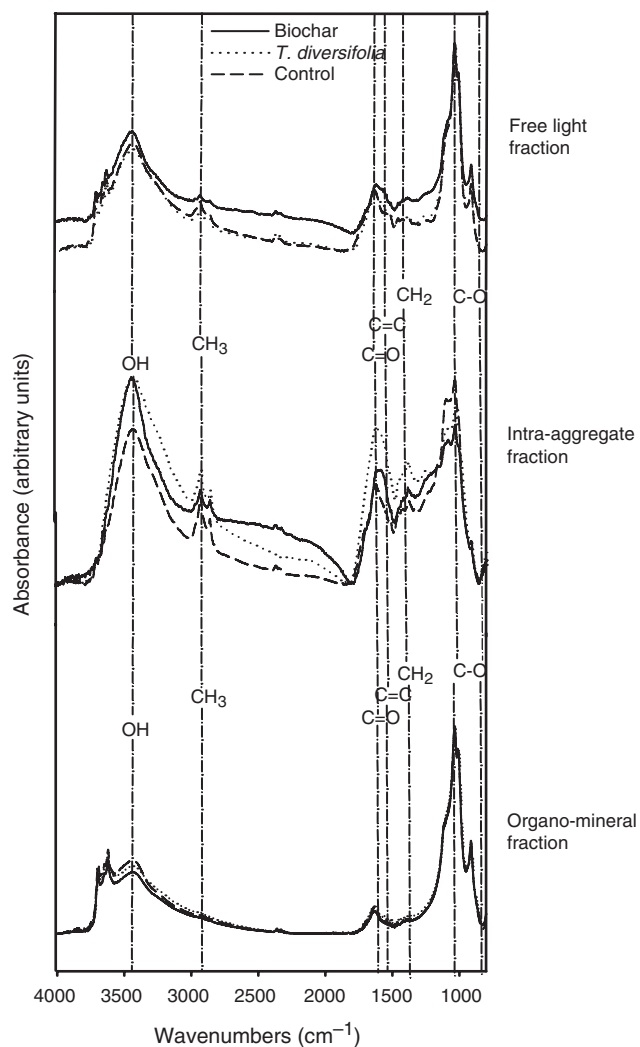


Fig. 3. FTIR analyses of soil fractions from the C-poor soils (105 years of continuous cereal cropping) as a function of the quality of added organic matter.

conspicuously depleted especially in the intra-aggregate fraction (Fig. 3).

Discussion

Pre-existing SOC and the stabilisation of added organic carbon

We observed lower absolute increases in C mineralisation after addition of biochar and *T. diversifolia* in the C-poor soils, which likely indicates that more C was stabilised compared with the C-rich soils. This was corroborated by a greater amount of C accrued in the more stabilised intra-aggregate fraction with decreasing SOC contents. Additionally, even without any OC addition, more C was lost from the C-rich soil than the C-poor soil at a rate of 0.14 kg/m².year, which coincides with a greater microbial biomass (Table 1). Similarly, existing studies in high C-containing soils have shown little or no increase in SOC with increased C inputs (Campbell *et al.* 1991; Paustian *et al.* 1997; Solberg *et al.* 1997; Gulde *et al.* 2008; Stewart *et al.* 2008). This

supports the saturation concept that soils containing large amounts of C stabilise less of the C in added organic matter than soils with low C contents (Hassink 1996; Six *et al.* 2002; Kimetu *et al.* 2009). Our observations differ from those made by Knoth (2004), who found that higher C stabilisation could be attained when OC is added to less-degraded soils with greater amounts of SOC. This may be explained by the low levels of aggregation in the coarse-silty mixed mesic Typic Haploxerolls studied by Knoth (2004); whereas, the Kenyan Ultisols studied here are more strongly aggregated. Lehmann *et al.* (2001) found that for a highly aggregated Xanthic Ferralsol in the central Amazon, the amount of organic C had no effect on aggregation. Loss of aggregation and therefore reduced capacity to stabilise added organic matter within aggregates may not have played a role at the current study sites.

Effects of added OM quality on the quantity and quality of stabilised C

Although the actual rate and degree of OC decomposition is controlled by the decomposer community and environmental conditions, the quality of the decomposing organic resource regulates the potential rate and the resultant end products of the decomposition process (Giller and Cadisch 1997). The quality of added OC may influence not only the amount of undecomposed OC but also the fluxes of decomposing OC between soil fractions. Application of biochar increased total OC contents as well as OC contents in the intra-aggregate fraction to a greater extent than application of *T. diversifolia* with lower OC contents of the soil ($P < 0.0001$). When expressed as the proportion of OC increase per unit C respired, the proportion of OC found in the intra-aggregate fractions was 6.8 times greater after adding low-quality biochar than high-quality green manure averaged across the degradation gradient ($P = 0.042$; $n = 3$). Thus, we see that the OC remaining in the light fraction after adding biochar will be incorporated into the intra-aggregate and organo-mineral fractions that are typically interpreted as the more stable soil fractions (Six *et al.* 2002; Zimmermann *et al.* 2007). Therefore, low-quality biochar not only remained unprocessed but could be stabilised to a greater degree in soil in the long term. These observations agree with the conclusion made by Mapfumo *et al.* (2007), who indicate that low-quality organic resources increase the size of the free light fraction. This may be explained by a slower processing of added OC by microorganisms as shown by lower CO₂ evolution and therefore a greater opportunity for the added OC to be incorporated into aggregates and for sorption onto minerals. Alternatively, the more rapid C accrual in more stable SOC fractions may be explained with a more rapid incorporation of applied litter into stable soil fractions in soils that are rich in black C (Liang *et al.* 2010), which was also indicated by soils amended with biochar (Kuzyakov *et al.* 2009).

Our findings are, however, contrary to the observations by Wardle *et al.* (2008), who indicate that biochar can prime humus decomposition. The difference may be explained by the fact that those researchers carried out their work in high C-containing organic horizons, whereas in the present study the positive effect of biochar was clearly observed in the low C-containing mineral soils. Our results corroborate laboratory results by Kuzyakov *et al.* (2009) and Spokas *et al.* (2009), who in their incubation

experiments showed lower, rather than higher, CO₂ evolution after biochar addition to soils. Major *et al.* (2010) found greater emissions from biochar-applied plots that could be explained by greater primary productivity in their study sites. In our experiment, we excluded plant growth from the CO₂ chambers and primary productivity would not have been captured. This may explain why, in contrast to Major *et al.* (2010), we found a net reduction in CO₂ emissions.

The greater abundance of aromatic C in all fractions including the organo-mineral fraction with additions of the stable biochar suggests that the stabilised organic matter bears a chemical signature of the added OM, in this case aromatic C. This greater stabilisation of biochar was more pronounced in the C-poor soils with lower SOC contents. This can be attributed to the lower total amounts of C mineralised with biochar additions than without, which may indicate that the pre-existing SOC was mineralised to a lesser degree. As demonstrated by Liang *et al.* (2010) and Neill (2007), microbial populations could be even higher in soils rich in black C. Thus, this decrease in microbial activity is unlikely to be attributed to a decrease in microbial biomass. Therefore, the other possible explanation for this phenomenon could be the reduction in the accessibility of C by the microbes and enzymes, possibly due to sorption of the labile C to the added biochar (Pietikäinen *et al.* 2000; Lehmann *et al.* 2005). A second explanation could be a possible, more-rapid stabilisation of native SOC (non-biochar C) into intra-aggregate and organo-mineral fractions (Liang *et al.* 2010).

Conclusions

In this study we demonstrate that stabilisation of biochar is an important process in soil that influences its residence time, and is therefore dependent on existing soil organic matter. The data in this study indicate that not only the stability but also the stabilisation of biochar exceeds that of a labile organic matter addition such as green manure. Greater increases in total SOC and greater stabilisation of added C could be attained with biochar due not only to greater amounts of undecomposed OC but to greater incorporation into more stable soil fractions. Biochar resulted in reduced mineralisation losses of existing soil C, which is most likely a result of greater stabilisation of the pre-existing C. The mechanisms remain unclear and require further studies. Particularly, the mechanisms by which soil mineralogy and texture interact with biochar and affect cycling of pre-existing soil C and microbial communities warrants further research.

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