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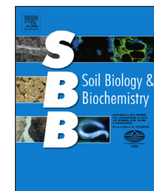

Biochar stability in soil: Decomposition during eight years and transformation as assessed by compound-specific ^{14}C analysis

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A B S T R A C T

Stability and transformation products of incomplete combustion of vegetation or fossil fuel, frequently called pyrogenic or black carbon and of biochar in soil, remains unknown mainly because of their high recalcitrance compared to other natural substances. Therefore, direct estimations of biochar decomposition and transformations are difficult because 1) changes are too small for any relevant experimental period and 2) due to methodological constraints (ambiguity of the origin of investigated compounds). We used ^{14}C-labeled biochar to trace its decomposition to CO_2 during 8.5 years and transformation of its chemical compounds: neutral lipids, glycolipids, phospholipids, polysaccharides and benzenepolycarboxylic acids (BPCA).

^{14}C-labeled biochar was produced by charring ^{14}C-labeled *Lolium* residues. We incubated the ^{14}C-labeled biochar in a Haplic Luvisol and in loess for 8.5 years under controlled conditions. In total only about 6% of initially added biochar were mineralized to CO_2 during the 8.5 years. This is probably the slowest decomposition obtained experimentally for any natural organic compound. The biochar decomposition rates estimated by $^{14}CO_2$ efflux between the 5th and 8th years were of 7×10^{-4} % per day. This corresponds to less than 0.3% per year under optimal conditions and is about 2.5 times slower as reported from the previous shorter study (3.5 years).

After 3.5 years of incubation, we analyzed ^{14}C in dissolved organic matter, microbial biomass, and sequentially extracted neutral lipids, glycolipids, phospholipids, polysaccharides and BPCA. Biochar-derived C (^{14}C) in microbial biomass ranged between 0.3 and 0.95% of the ^{14}C input. Biochar-derived C in all lipid fractions was less than 1%. Over 3.5 years, glycolipids and phospholipids were decomposed 1.6 times faster (23% of their initial content per year) compared to neutral lipids (15% $year^{-1}$). Polysaccharides contributed ca. 17% of the ^{14}C activity in biochar. The highest portion of ^{14}C in the initial biochar (87%) was in BPCA decreasing only 7% over 3.5 years. Condensed aromatic moieties were the most stable fraction compared to all other biochar compounds and the high portion of BPCA in biochar explains its very high stability and its contribution to long-term C sequestration in soil.

Our new approach for analysis of biochar stability combines ^{14}C-labeled biochar with ^{14}C determination in chemical fractions allowed tracing of transformation products not only in released CO_2 and in microbial biomass, but also evaluation of decomposition of various biochar compounds with different chemical properties.

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1. Introduction

Pyrogenic carbon produced by incomplete combustion of plant biomass or fossil fuel (Kuhlbusch, 1998) is ubiquitous in soils (Schmidt and Noack, 2000) and marine sediments (Masiello and Druffel, 1998). The interest in pyrogenic C is mainly connected with its importance for the global C cycle (Kuhlbusch, 1998; Forbes et al., 2006) and with its potential role as a C sink in soils and sediments for long periods of time, because its microbial decomposition and chemical transformation is apparently very slow. Recently, the interest was strongly raised because of biochar applications and “Terra Preta” phenomenon (Glaser et al., 2001), which also has very high stability and additionally may strongly

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improve soil fertility. The chemical and biological inertness of pyrogenic C and biochar is mainly based on: 1) high resistance to a range of chemical oxidants, 2) preservation for long periods in geological records, and 3) existence at soil depths where the residence time exceeds millennia (Forbes et al., 2006).

Various descriptive methods of pyrogenic C and biochar identification in soils (Schmidt and Noack, 2000; Preston and Schmidt, 2006; Hammes et al., 2007; Leifeld, 2007) and of assessing its physical and chemical structure (Glaser et al., 1998; Schmidt et al., 2002; Brodowski et al., 2005) have been developed. Improved approaches were used to estimate burial periods revealing fire history (Ballentine et al., 1998; Dai et al., 2005), allow pyrogenic C detection in soils as well as the evaluation of black C sources (Glaser and Knorr, 2008).

In contrast to the descriptive methods, only very few studies described transformation processes, especially the rates of transformations including complete mineralization of pyrogenic C to CO_2. Based on scanning electron microscopy (SEM) coupled to energy-dispersive X-ray spectrometry (EDX), Glaser et al. (2000) and Brodowski et al. (2005) showed that pyrogenic C is slowly oxidized and may be bound to mineral particles. However, this approach is not applicable to the oxidation periods necessary to evaluate transformation rates of pyrogenic C. Comparing historical samples and newly produced pyrogenic C, Cheng et al. (2008a) showed its substantial oxidation during 130 years and related the oxidation intensity to the mean annual temperature.

The lack of studies estimating process rates is explainable by biochar stability – by inertness of pyrogenic C for biological and chemical reactions (Forbes et al., 2006; Preston and Schmidt, 2006) especially oxidation (Hammes et al., 2007; Cheng et al., 2008a). This would entail very long periods necessary to obtain measurable transformations, or indirect approaches i.e. false time series with their restrictions should be used. All studies assumed very slow transformation rates and suggested a very long mean residence time (MRT) of biochar in soils. Direct evidence of long MRT is based mainly on $\Delta^{14}C$ measurements of pyrogenic C and biochar in soils (Schmidt et al., 2002; Gavin et al., 2003) and marine sediments (Masiello and Druffel, 1998). As the microbial activity in sediments is extremely low, the MRT observed there cannot be transferred to soils.

There are only very few studies estimating biochar decomposition rates in soils (Kuzyakov et al., 2009; Hilscher and Knicker, 2011). This is because the changes of biochar content are too small for any practical experimental period (months to few years). Many studies estimating the decomposition rates of substances with short and medium MRT in soils are based on changes of CO_2 efflux after substance addition. This approach is unsuitable to estimate biochar decomposition because of the much higher contributions of soil organic matter and plant residues mineralization to the CO_2 compared to biochar.

To solve this problem, we used a new approach – ^{14}C labeling of biochar (Kuzyakov et al., 2009). We produced ^{14}C-labeled biochar from shoot litter of *Lolium perenne* labeled with ^{14}C, mixed this biochar with soil or loess, and incubated for 3.2 years. Extremely slow $^{14}CO_2$ release of about 0.5% C per year was observed after 3.2 years incubation under optimal conditions. These rates extrapolated to field conditions showed MRT of biochar of about 2000 years. To our knowledge, this was the first experimental evidence of very slow decomposition rates of biochar. Furthermore, we showed that addition of a primer or aggregate destruction simulated biochar decomposition for short periods of few weeks, but had no effect over years.

Considering very slow decomposition rate of biochar as well as its continuous decrease, we prolonged this incubation for additional 5 years. Therefore, the incubation was ongoing in total 8.5 years. Furthermore, we made chemical fractionation of initial biochar and biochar remained in soil after 3.5 years of incubation and analyzed ^{14}C in dissolved organic matter, microbial biomass, three lipid fractions, polysaccharides and condensed aromatic moieties to evaluate what are the most stable biochar compounds.

2. Material and methods

2.1. Soil and loess samples

The soil was sampled from the Ap horizon of a loamy Haplic Luvisol (long-term experimental station Karlshof of Hohenheim University, Stuttgart, Germany). The soil originated from loess; it contains no $CaCO_3$ and has the following characteristics: pH 6.0, TOC 1.2%, N_t 0.13%, clay 23%, silt 73%, and sand 4.4%. The soil was air-dried, sieved <2 mm, and 45 g dry soil were filled in the incubation jars.

Loess samples for the experiment were taken from 15 m depth of an open cast mine at Nussloch, SW Germany (49.19 °N, 8.43 °E, 217 m asl.). Detailed description of loess is presented in Kuzyakov et al. (2006). 44.1 g of loess (air-dried and sieved <2 mm) were thoroughly mixed with 0.9 g loamy Haplic Luvisol to introduce microorganisms and were filled in the incubation jars. The soil-loess mixture contained 1.24 mg TOC g^{-1}. This loess-soil mixture is termed here as loess.

2.2. ^{14}C labeled biochar

The ^{14}C labeled biochar was produced from shoots of *L. perenne* that was uniformly labeled with ^{14}C. The litter remains after a cutting experiment, in which the *Lolium* shoots were labeled 7 times during 2 months (Kuzyakov et al., 2002). Nine grams of shoot litter dried at 60 °C were ball milled (MM2, Fa Retsch) for 1 min and put in the muffle furnace in closed, thick-walled steel crucibles (i∅/o∅ = 25.5/33.3 mm × 650 mm high, steel walls 3.9 mm). The charring of ^{14}C-labeled litter was done by slowly heating during 4.5 h from 20 to 400 °C following by 13 h at 400 °C (details in Kuzyakov et al., 2009). The produced biochar was pitch black and contained 55% C and 2.5% N. It was mixed again and 108 mg of biochar with a ^{14}C specific activity of 13.3 Bq mg^{-1} were added to each incubation jar containing 45 g of soil or of loess. This corresponds to 20% and 200% of TOC in soil and loess, respectively and a total ^{14}C activity of 1.44 kBq per jar. Soil or loess were thoroughly mixed with added biochar before incubation.

2.3. Experimental layout and incubation conditions

The samples of 45 g soil or loess material with or without biochar were incubated in 250-mL Schott jars for 8.5 years at 20 °C and 70% of water holding capacity by addition of 9.45 and 8.82 ml distilled water to soil and loess, respectively. During the incubation, the CO_2 evolved from the soil or loess was trapped in 3 ml of 1.0 M NaOH solution placed in small caps into the incubation jars. Periodically, the NaOH with trapped CO_2 was sampled and replaced with new NaOH. During the 8.5 years period, the NaOH trap was exchanged 59 times. The soil moisture was controlled gravimetrically and water was added if necessary.

At day 624 (1.7 years) and at day 1257 (3.5 years), incorporation of ^{14}C from biochar into microbial biomass and dissolved organic carbon (DOC) was determined by the fumigation–extraction method by destructive sampling of replicate Schott jars. Additionally, at day 1257 (3.5 years), the ^{14}C activity was analyzed in following fractions: total lipids, neutral lipids, glycolipids, phospholipids (PLFA), polysaccharides, benzenepolycarboxylic acids (BPCA) and soil residue (Fig. 1).

2.4. Sample analysis

2.4.1. Analysis of ^{14}C activity in CO_2

The ^{14}C activity of CO_2 trapped in a NaOH solution was measured by scintillation counting (Beckmann 6500, Beckmann, Germany) in

2 ml aliquots with 4 ml of the scintillation cocktail. To ensure high precision of ^{14}C counting, the radioactivity background was measured every 10 samples and measuring time was set at least for 30 min (up to 120 min) for each sample.

The total CO_2 trapped in the NaOH solution was measured by titration of 1 ml aliquot with 0.2 M HCl (Werth and Kuzyakov, 2006). Total C and N content of the soil, loess, *Lolium* shoots and biochar were measured using a LECO elemental analyzer.

2.4.2. Analysis of ^{14}C activity in microbial biomass and in dissolved organic carbon

Soil microbial biomass was determined by the chloroform fumigation–extraction method. One gram of fresh soil or loess was extracted with 4 ml of 0.05 M K_2SO_4 solution. Another 1 g of fresh soil was first fumigated with chloroform for 24 h and then extracted in the same way. After shaking (1 h at 200 rpm) and centrifugation (3000 rpm for 10 min) the solution was filtered through a ceramic vacuum filter. C concentrations were analyzed on a 2100 TOC/TIC analyzer (Analytik Jena, Germany). Microbial biomass C content was calculated by using a kEC value of 0.45 for C and ^{14}C and is presented as mg C in 1 g of dry soil (for total C) and as percentage of the added biochar (for ^{14}C).

2.4.3. Analysis of ^{14}C activity in lipid fractions, polysaccharides and benzenepolycarboxylic acids (BPCA)

Five grams of soil were soxhlet-extracted with 100 ml dichloromethane and 50 ml methanol for 36 h. The extract was separated into neutral lipids, phospholipids and glycolipids by sequential liquid–liquid extraction with chloroform, methanol and acetone, respectively (Fig. 1, Apostel et al., 2013). After extraction and purification of lipids, the ^{14}C activity of the three lipid fractions was analyzed (see above).

The residue after lipid extraction was dried at 40 °C, transferred into a 20-ml hydrolysis flask and hydrolyzed with 10 ml 4 M trifluoroacetic acid (TFA) for 4 h (Amelung et al., 1996; Spielvogel et al., 2007). After filtration with a glass fiber filter, TFA was removed by rotary evaporation (Eder et al., 2010). Interfering humic substances were removed by adsorption (XAD-7) and cation chromatography (Dowex 50 WX8). After extraction and purification of polysaccharides, their ^{14}C activity was analyzed (see above).

The residue of the TFA hydrolysis was dried at 40 °C and transferred into a quartz digestion tube. Benzenepolycarboxylic acids were produced from condensed aromatic moieties of biochar by high pressure digestion with 65% nitric acid for 8 h at 170 °C (Glaser et al., 1998). Digestates were filtered by ash-free cellulose filters and purified with Dowex 50 WX8. After extraction and purification of BPCA, their ^{14}C activity was analyzed on scintillation counter (see above).

2.5. Calculations and statistics

The biochar decomposition rates ($^{14}CO_2$) were measured for soil and loess in 8 independent replicates. The biochar metabolites were analyzed with 4 replicates with subsequent ^{14}C measurements. All results were re-calculated as percentage from the ^{14}C input activity (100%). The significance of differences between the soil and loess was calculated by ANOVA. The standard error of means is presented in figures.

3. Results

3.1. Biochar decomposition to CO_2

The decomposition of biochar was estimated based on the $^{14}CO_2$ efflux from soil (Fig. 2), because the changes in the total CO_2 efflux

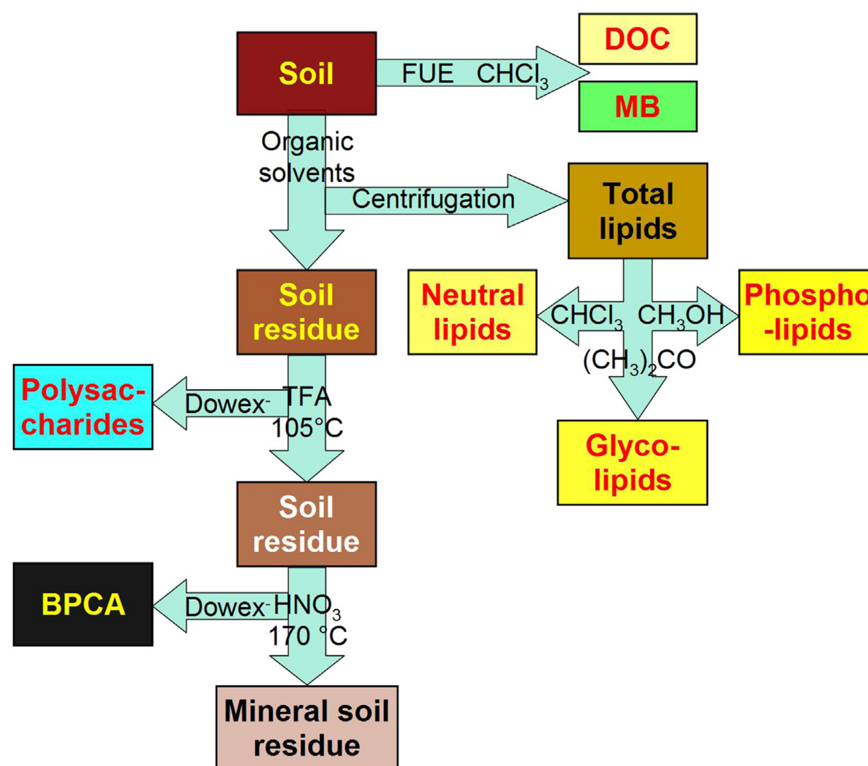

Fig. 1. Fractionation scheme of biochar. Detailed explanations of the methods in text. FUE: fumigation extraction, MB: microbial biomass, DOC: dissolved organic carbon. ^{14}C was analyzed in all final fractions as well as in total lipids.

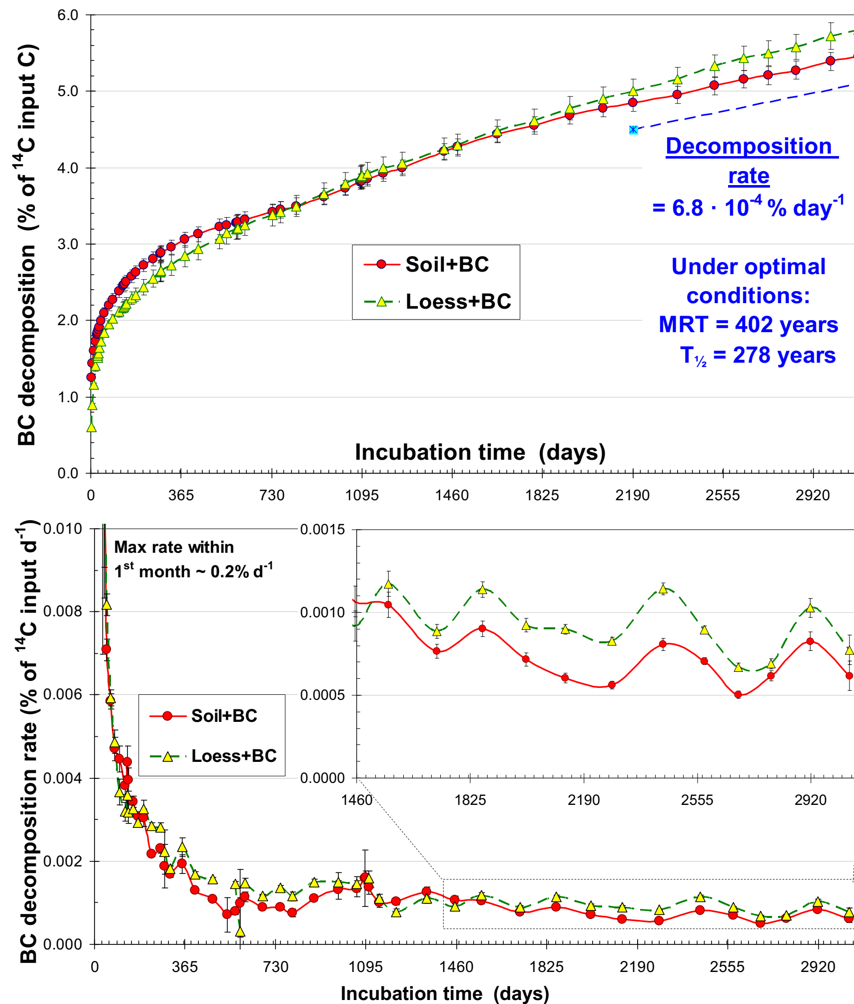

Fig. 2. Biochar (BC) mineralization in soil and loess during 8.5 years analyzed based on $^{14}CO_2$ efflux. Top: cumulative $^{14}CO_2$ efflux; bottom: biochar mineralization rates. The inset shows magnification of biochar decomposition rates between years 4 and 8. The blue dashed line on the top right shows the average decomposition between year 6 and 8.5 ($\sim 6.8 \times 10^{-4}$ % day^{-1}). Error bars show standard errors (±SE, $n = 8$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

after biochar addition were too small to allow relevant conclusions (see Fig. 2 from Kuzyakov et al., 2009). The $^{14}CO_2$ efflux showed a very slow biochar mineralization. Less than 6% of the ^{14}C added as biochar were released as $^{14}CO_2$ during 8.5 years, and most of it was released during the first 2 years (Fig. 2 top). Biochar mineralization rates strongly decreased during the incubation (Fig. 2, bottom). After one year, the biochar mineralization decreased by more than one order of magnitude and amounted to 1.2×10^{-3} and 1.6×10^{-3} % d^{-1} for soil and loess, respectively. After five years, the biochar decomposition rate was about 1.4×10^{-3} % d^{-1}. After 8.5 years, the decomposition rate was 0.7×10^{-3} % d^{-1}, slightly less in soil compared to that in loess (see inset in Fig 2, bottom). Based on the biochar mineralization dynamics (Fig. 3), we expect that the mineralization will further decrease.

3.2. Incorporation of biochar C into microbial biomass

Microbial biomass measured by the fumigation-extraction approach 624 d (1.7 years) after the start of incubation was higher in the soil (740 μg g^{-1}) than in the loess (418 μg g^{-1}) (Fig. 3, top). Even though more than 96% of the added biochar remained in the soil after 1.7 years, the ^{14}C incorporation into microbial biomass was 2.6 and 1.5% of ^{14}C input for soil and loess, respectively (Fig. 3, top). The relative incorporation (^{14}C/C ratio) of biochar into microbial biomass was similar in soil and loess (data not shown).

Because of absence of C input during 8.5 years, the total amount of microbial C decreased by about 85–90% between 1.7 and 3.5 years for soil and loess (Fig. 3). Also the ^{14}C originated from biochar in microbial biomass decreased from year 1.7 to year 3.5 (Fig. 3). However, the ^{14}C from biochar in microbial biomass decreased much less than that of total C reflecting the fact that decomposition of biochar compounds even in microbial biomass is slower than that of other native organics.

Considering the detection limit of about 0.01% of initial biochar, no ^{14}C was measured in DOC extracted from soil or loess after 1.7 and 3.5 years of incubation.

3.3. Lipid fractions within biochar

Because produced biochar may contain lipids, not only the soil samples after 3.5 years incubation, but also the initial biochar was analyzed and ^{14}C activity was measured in all fractions. The initial biochar contained about 4.5% lipids with strong domination of glycolipids and phospholipids (Fig. 4). These two lipid fractions were strongly decomposed during 3.5 years, so that their contribution decreased below 0.5% and was similar in soil and loess. The

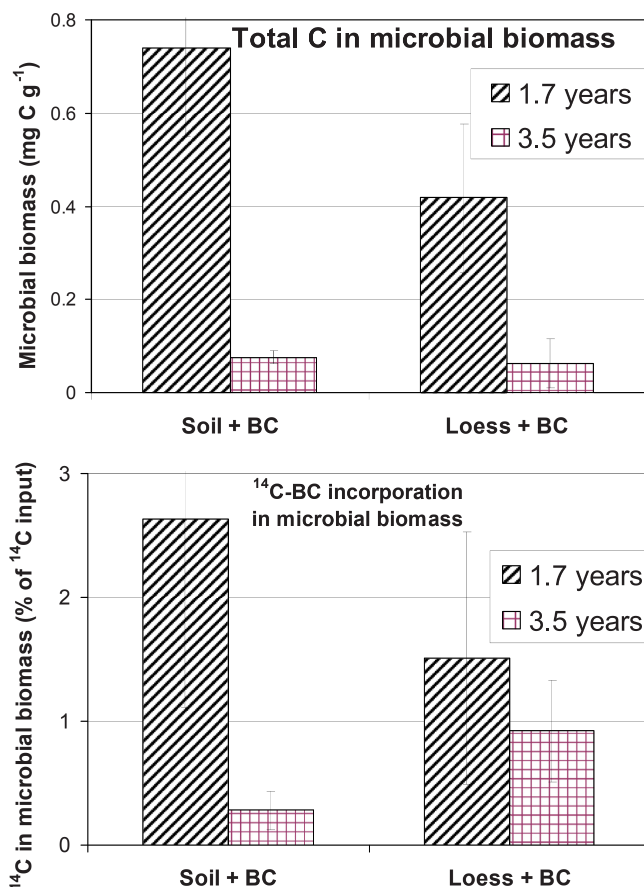

Fig. 3. Incorporation of ^{14}C from biochar (BC) into soil microbial biomass (MB) after 1.7 years (624 days) and 3.5 years (1277 days) of incubation in soil and loess. Top: Microbial biomass C content; Bottom: ^{14}C from biochar in microbial biomass. Error bars show standard errors ($n = 4$).

relative decomposition of neutral lipids was much slower, especially in loess (Fig. 4). After 3.5 years, less than 0.1% of initial biochar consisted of neutral lipids in soil. Generally, the decomposition of all lipid fractions from biochar was much faster than the decomposition of total biochar (Fig. 4).

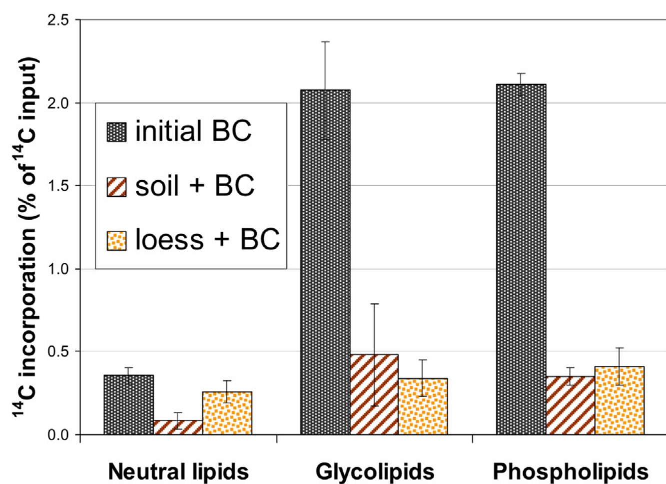

Fig. 4. ^{14}C from biochar (BC) in three lipid fractions: Neutral lipids, glycolipids and phospholipids after 3.5 years incubation in soil and loess. The “initial BC” represents the lipid content in the biochar before addition to the soil. Error bars show standard errors ($n = 4$).

3.4. ^{14}C from biochar in polysaccharides and condensed aromatic moieties

Between 15 and 18% of ^{14}C before and after 3.5 years of incubation was accounted in polysaccharides (Fig. 5). For this fraction the highest variation of ^{14}C was observed. Therefore, it was not possible to conclude about changes of ^{14}C in polysaccharides during 3.5 years.

Nearly 80% of ^{14}C remained in BPCA after 3.5 years of biochar incubation without any differences between soil and loess (Fig. 5). This is just 7% less than ^{14}C in BPCA in the initial biochar added to the soil. This is a smaller relative loss than for all other fractions extracted from biochar and corresponds to the decrease of ^{14}C in total biochar.

4. Discussion

4.1. Suitability of ^{14}C labeling to study biochar transformation

Five years ago, we introduced the ^{14}C labeling approach for direct estimation of biochar stability by its decomposition to CO_2 (Kuzyakov et al., 2009). Because biochar transformation in soils and sediments is extremely slow (Preston and Schmidt, 2006), common methods based on changes of biochar content as well on total CO_2 efflux will be unsuccessful, at least for studies, in which the labile fraction of biochar is already degraded. Although the specific ^{14}C activity of the produced biochar was low (13.3 Bq mg^{-1}), it was sufficient to estimate biochar decomposition rates of 7×10^{-6} d^{-1} ($= 7 \times 10^{-4}$ % d^{-1}). Such a high sensitivity allowed a precise analysis of chemical transformations and of biochar decomposition against the background of high CO_2 fluxes from other sources.

Biochar with a shifted $\delta^{13}C$ signature has been tested to evaluate its origin. By using ^{13}C labels at natural abundance levels, Glaser and Knorr (2008) found that up to 25% of black carbon in soils may be produced *in situ* by biotic processes, without fire or charring. However, they could not investigate the stability of this biogenic black carbon. Considering the very slow biochar decomposition, very low differences of $\delta^{13}C$ between the end members, as well as large uncertainties of ^{13}C fractionation by decomposition (Werth and Kuzyakov, 2010), the ^{13}C natural abundance is inappropriate for estimating biochar decomposition rates.

Recent studies showed that biochar decomposition can be estimated not only using ^{14}C, but also using biochar produced from

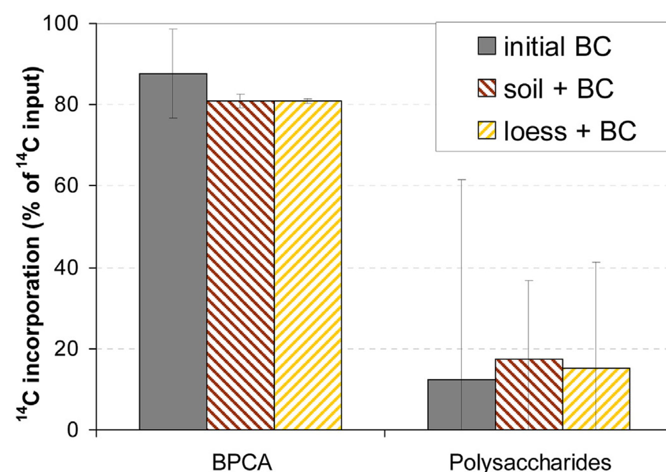

Fig. 5. ^{14}C from biochar (BC) in benzenepolycarboxylic acids (BPCA) and polysaccharides after 3.5 years incubation in soil and loess. The “initial BC” represents the content in the biochar before addition to the soil. Error bars show standard errors ($n = 4$).

^{13}C-labeled plant residues (Hilscher and Knicker, 2011). This required a high ^{13}C enrichment in the biochar and therefore, in the plant material source to obtain sensitivity comparable to that by ^{14}C. This is difficult because $^{13}CO_2$ with high enrichment should be provided to the plants throughout the growth period (continuous $^{13}CO_2$ labeling). In contrast to ^{13}C, the production of biochar with a much higher specific ^{14}C activity as used in our study is a comparatively simple issue and may further increase the sensitivity of the approach by 3–5 orders of magnitude.

Further advantage of the suggested approach is that uniform labeling of plants is not a prerequisite to produce uniformly labeled biochar. Because most of the C atoms in biochar build its aromatic structure (Chatterjee et al., 2012), they are identical concerning ^{14}C (or ^{13}C) positions. Furthermore, charring the litter leads to ^{14}C (or ^{13}C) redistribution, and consequently uniformly labeling of produced biochar. Therefore, even one pulse labeling (with highly enriched $^{14}CO_2$) of plants producing non-uniformly labeled litter should be sufficient to produce uniformly labeled biochar.

Application of ^{14}C- and/or ^{13}C-labeled biochar opens a new way to trace not only the biochar itself, but also its transformation products. Thus, biochar-derived C can be traced in microorganisms, dissolved organic matter and organic matter fractions. It can be coupled with physical fractions such as particle size or density fractionation (Hilscher and Knicker, 2011).

4.2. Biochar decomposition to CO_2 and its mean residence time in soil

As expected, biochar decomposition was very slow. Similar to most other substances such as sugars, lipids, lignin etc., the biochar decomposition strongly decreased during time. During 8.5 years, the mineralization rates decreased nearly by two orders of magnitude. This reflects not only potential protection of biochar particles within soil aggregates, but mainly continuous preferential utilization of compounds according their degradability (Cheng et al., 2008b; Kuzyakov et al., 2009; Blagodatskaya et al., 2011). Biochar consists of a broad range of substances of different C condensation, aromatics, oxygen and nitrogen content, etc. (Preston and Schmidt, 2006; Hammes et al., 2007; Hilscher and Knicker, 2011). The organics with relative fast decomposition rates were exhausted only after 1.5–2 years (Fig. 2). This clearly shows that shorter experiments (few months) cannot adequately reflect the biochar decomposition.

Biochar decomposition kinetics within the first 3 years was analyzed earlier (Kuzyakov et al., 2009). Therefore, here we focus on the decomposition during the last 3–4 years (Fig. 2 bottom, see the inset). Biochar decomposition rates stabilized at the level of 7×10^{-4} % per day. It was slightly lower in soil compared to loess. We explain this by two reasons: 1) Faster biochar mineralization in soil compared to loess during the first two years led to smaller amounts of remaining relatively labile ^{14}C-labeled biochar components in the soil. 2) Other more easily degradable organics compared to biochar are present in soil and consequently, microorganisms in soil prefer to use these (unlabeled) substrates compared to biochar (Blagodatskaya et al., 2009). Because the total $^{14}CO_2$ efflux over 8.5 years was similar in soil and in loess, we conclude that not the soil properties (C or N contents, microbial biomass, etc.), but rather biochar characteristics are crucial for its mineralization.

The mean residence time (MRT) was calculated as reciprocal to decomposition rates (Derrien and Amelung, 2011; Kuzyakov, 2011). Thus, the MRT of biochar under optimal incubation conditions during the period 5–8.5 years is about 400 years. In the previous study we extrapolated this MRT obtained under optimal conditions to the field conditions based on the Biological Active Time (BAT) approach (Franko et al., 1997). If we use the same BAT approach here, the MRT under field conditions will reach ~4000 years. The BAT approach was elaborated for studies over weeks and months (Franko et al., 1997) and it is probably very rough and actually speculative extrapolation for periods over decades and centuries. Although we are aware about very high uncertainties of our extrapolation, it is in the range of MRTs estimated for biochar by radiocarbon dating, e.g. in European Chernozems (1160–5040 years, Schmidt et al., 2002) or in ocean sediments (2400–13,900 years, Masiello and Druffel, 1998).

However, there is a discrepancy between the results from this and other studies showing millennial lifetimes, versus some field studies showing shorter turnover times of centuries. Hammes et al. (2008) found significant reduction of black carbon content in a 100-years false time series of soils in Russian steppes. They found 2.5 kg m^{-2} of black carbon corresponding to 7–10% of total organic C in 1900, which decreased for 25% over one century translating into a turnover of about 180–540 years (mean 290 years). We speculate that this comparatively fast turnover is connected with decomposition of non-pyrogenic, but of biogenic black carbon (Glaser and Knorr, 2008), which probably has much faster turnover rate. Singh et al. (2012) used two modeling approaches to estimate biochar degradation based on data from 16 studies ($n = 54$) ranging from decadal to centennial time scale, varying in initial plant biomass type, pyrolysis temperature, and experimental conditions. They revealed an average turnover time using a one-pool approach of 88 years, and the best estimate using a two-pool approach was 3 years for a fast-cycling pool and 870 years for a slow-cycling pool.

Various factors absent by incubation in jars may accelerate biochar decomposition under field conditions. These factors include 1) freezing/thawing cycles, which may disperse biochar particles and increase their active surface, 2) drying/wetting, which may increase the local concentration of enzymes at the biochar surface, 3) presence of living roots may stimulate microbial activity and increase C turnover (Kuzyakov et al., 2007; Pausch et al., 2013), and 4) soil mixing by animals and aggregate destruction by living roots as well as by drying/wetting and freezing/thawing cycles. These effects on MRT of biochar should be proven in details in further studies, especially for biochar already under decomposition for some years.

4.3. Biochar incorporation into soil microbial biomass

This study appears to be one of the first showing direct incorporation of biochar-C into microbial biomass and the first one for biochar decomposition over 3.5 years. The biochar-C incorporated into microbial biomass shows the portion being used by microorganisms at the sampling time. Between 1.5 and 2.6% of the remaining 96% biochar were recovered in microbial biomass after 1.7 years. After 3.5 years, 0.3 and 0.9% of initial ^{14}C were recovered in microbial biomass. This shows a low microbial availability of biochar and indirectly confirms that biochar will be decomposed mainly by co-metabolism (Hamer et al., 2004; Kuzyakov et al., 2009) and is of negligible importance as a C source for microorganisms.

Comparison of the decrease of ^{14}C from biochar and of total C in microbial biomass between 1.7 and 3.5 years clearly shows that ^{14}C decrease within microorganisms is on average 1/3 slower. This clearly reflects that C from biochar will be used by microorganisms less intensively compared to C from soil organic matter. This is also an indirect confirmation of very low microbial availability of biochar. We assume that mainly lipids and polysaccharides from initial biochar were incorporated into microorganisms.

Similarly as after 1.7 years of biochar decomposition (Kuzyakov et al., 2009), we found no ^{14}C from biochar in DOC after 3.5 years. This contrasts with the interpretation of result from Brazil (Dittmar et al., 2012) or reviewed on a global basis (Jaffé, 2013). They showed

that DOC is composed of about 7–10% of condensed aromatic moieties and interpret this as dissolved black carbon. All soil organic matter pools contain aromatic moieties at various percentage, even in soils that never obtained pyrogenic C. According to the sensitivity of our approach (based on ^{14}C-specific activity) the ^{14}C incorporation of biochar into DOC is less than about 0.01% of initial biochar content. This estimation is close to the results of a batch experiment, where only ~0.15% of charcoal were found to be soluble (Abiven et al., 2011).

4.4. Decomposition of biochar compounds

Initial biochar consisted of 4.5 ± 0.3% lipids, 12% polysaccharides and 87.7 ± 11% of condensed aromatic moieties. This confirms that biochar produced by litter charring has very high level of polyaromatic structures (high BPCA) corresponding to natural pyrogenic C and that produced in other studies (Schimmelpfennig and Glaser, 2012).

More than 80% of glycolipids and phospholipids were decomposed within 3.5 years (Fig. 4) corroborating their relative instability. Neutral lipids showed higher stability as their content decreased only for about two times over 3.5 years. The absence of functional groups explains higher stability of neutral lipids and therefore, they may accumulate in soil relative to other compounds (Koegel-Knabner et al., 2005). Therefore, neutral lipids are frequently used as medium term biomarker in soil or long-term biomarkers in aquatic sediments (Gleixner et al., 2002; Glaser and Zech, 2005).

Neutral lipids consist of alkanes and aliphatic carboxylic acids, but in this study we did not separate them. The decomposition of carboxylic acids is much faster compared to n-alkanes and n-alkanes are used as long-term biomarkers because of their relative recalcitrance (Wiesenberg et al., 2004). Therefore, we assume that at least a part of the decrease of neutral lipids observed in this study (Fig. 4) is connected with decomposition of carboxylic acids. Considering the decomposition of about half of the amount of neutral lipids during 3.5 years, and probably changes of their composition, the applicability of neutral lipids as medium- and long-term biomarkers should be revised.

Another reason for the presence of the three lipid fractions after 3.5 years could be their production by biochar transformation. If this is the case, the actual decomposition of all lipids is even (much) faster than measured according to their ^{14}C decrease. In this study, we have not differentiated whether the lipids are remaining from decomposition of their initial content or they were newly produced from other ^{14}C-labeled compounds during the incubation.

The biggest part of initial biochar consists of condensed aromatic moieties as assessed by BPCA extraction. Their decomposition was very small over 3.5 years – only about 7%, but was not significant because of high variation of BPCA extraction from initial biochar. Hammes et al. (2008) showed a relatively fast turnover of black carbon in Chernozems on a centennial scale using a false time series under field conditions. Therefore, further studies are urgently needed to show whether aromatic moieties of biochar are stable or not under field conditions.

We cannot conclude about the decomposition of polysaccharides because of high variability of their extraction from biochar. Nevertheless, we conclude that after 3.5 years nearly all remaining biochar consists of condensed aromatic moieties and that only 6% of the original biochar was mineralized within 8.5 years.

4.5. Relative stability and decomposition of biochar compounds

Considering degradation of chemical fractions of biochar, we can calculate and compare their relative decomposition during one year

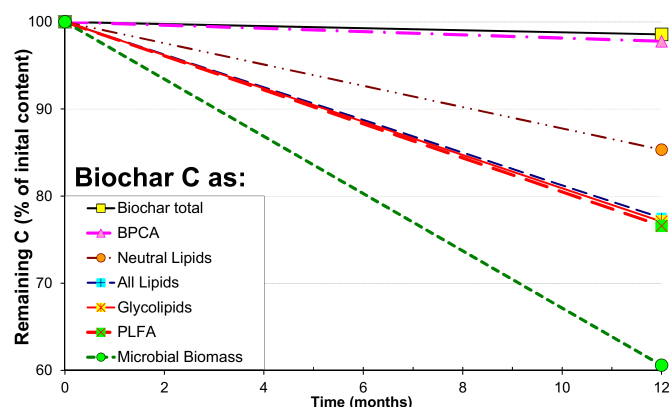

Fig. 6. The percentage of C remaining by decomposition of individual biochar compounds during one year of incubation under optimal conditions. The values are presented as relative data (from 100% of each compound class at time 0) because of the compounds have very different content in the initial biochar. The points present average values for soil and loess and were calculated assuming the same decomposition rate during 3.5 years.

(Fig. 6). Because of small portions decomposed over 3.5 years, we can assume linear decomposition. The highest decomposition rate of C from biochar was observed in microbial biomass, because microorganisms used the most easily available C from other recalcitrant components and because of relatively high turnover of microbial C (Blagodatskaya et al., 2011).

Over one year, about 25% of all lipids were mineralized to CO_2. Despite the decomposition of neutral lipids was much slower (Fig. 6), their small contribution to total lipids in biochar had no significant effect on ^{14}C loss from total lipids. Consequently, the decrease of total lipid content in biochar corresponded to that of glycolipids and phospholipids.

The slowest decomposition of just 2.2% per year was observed for BPCA and because of their dominance, it was almost identical to the decomposition of total biochar (Fig. 6). Therefore, we conclude that the high recalcitrance of biochar is connected to its high content of BPCA and its extremely slow microbial decomposition.

5. Conclusions

Direct analysis of biochar decomposition in soil during 8.5 years under optimal conditions showed very slow rates of about 7×10^{-4} % d^{-1}, corresponding to a decomposition of about 0.26% per year. Considering the much slower decomposition under field conditions, we estimated the mean residence time of biochar in soils of temperate climates to be about 4000 years. We are aware that this extrapolation of decomposition rates obtained in laboratory to the field conditions is very speculative and need further confirmation.

The incorporation of biochar into microorganisms after 1.7 years varied between 1.5 and 2.6% of biochar-derived C, confirming our hypothesis on co-metabolic biochar decomposition. Despite strong decrease of biochar-originated compounds in microbial biomass between 1.7 and 3.5 years, this decrease was ~30% slower compared to the decline of total microbial C. This shows that even microbially utilized biochar compounds have much slower turnover within microorganisms compared to other C sources.

Phospholipids and glycolipids contained in initial biochar strongly decreased over 3.5 years, and their decomposition was 3–4 times faster compared to neutral lipids. After 3.5 years, the ^{14}C in all lipids was less than 1%. The initial biochar consisted to 87% of BPCA and their decrease (~7%) was not significant over 3.5 years.

The BPCA were the most stable fraction in biochar responsible for its very high chemical recalcitrance.

These results reflecting very slow decomposition of biochar, its microbial utilization and transformation were possible only because biochar was labeled with ^{14}C. We conclude that applying ^{14}C-labeled biochar opens new ways to identify and quantify very slow processes because of the high sensitivity and specificity of ^{14}C analysis. This approach also allows calculation of the C budget and analysis of decomposition rates of biochar compounds. ^{14}C-labeled biochar may also be used for evaluation and optimization of biochar analysis in soils and sediments.

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