Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling

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ABSTRACT

Incomplete combustion of organics such as vegetation or fossil fuel led to accumulation of charred products in the upper soil horizon. Such charred products, frequently called pyrogenic carbon or black carbon (BC), may act as an important long-term carbon (C) sink because its microbial decomposition and chemical transformation is probably very slow. Direct estimations of BC decomposition rates are absent because the BC content changes are too small for any relevant experimental period. Estimations based on CO_2 efflux are also unsuitable because the contribution of BC to CO_2 is too small compared to soil organic matter (SOM) and other sources.

We produced BC by charring ^{14}C labeled residues of perennial ryegrass (*Lolium perenne*). We then incubated this ^{14}C labeled BC in Ah of a Haplic Luvisol soil originated from loess or in loess for 3.2 years. The decomposition rates of BC were estimated based on [14]CO_2 sampled 44 times during the 3.2 years incubation period (1181 days). Additionally we introduced five repeated treatments with either 1) addition of glucose as an energy source for microorganisms to initiate cometabolic BC decomposition or 2) intensive mixing of the soil to check the effect of mechanical disturbance of aggregates on BC decomposition. Black carbon addition amounting to 20% of C_{org} of the soil or 200% of C_{org} of loess did not change total CO_2 efflux from the soil and slightly decreased it from the loess. This shows a very low BC contribution to recent CO_2 fluxes. The decomposition rates of BC calculated based on ^{14}C in CO_2 were similar in soil and in loess and amounted to $1.36 \; 10^{-5} \, d^{-1}$ ($=1.36 \; 10^{-3}\% \, d^{-1}$). This corresponds to a decomposition of about 0.5% BC per year under optimal conditions. Considering about 10 times slower decomposition of BC under natural conditions, the mean residence time (MRT) of BC is about 2000 years, and the half-life is about 1400 years. Considering the short duration of the incubation and the typical decreasing decomposition rates with time, we conclude that the MRT of BC in soils is in the range of millennia.

The strong increase in BC decomposition rates (up to 6 times) after adding glucose and the decrease of this stimulation after 2 weeks in the soil (and after 3 months in loess) allowed us to conclude cometabolic BC decomposition. This was supported by higher stimulation of BC decomposition by glucose addition compared to mechanical disturbance as well as higher glucose effects in loess compared to the soil. The effect of mechanical disturbance was over within 2 weeks. The incorporation of BC into microorganisms (fumigation/extraction) after 624 days of incubation amounted to 2.6 and 1.5% of ^{14}C input into soil and loess, respectively. The amount of BC in dissolved organic carbon (DOC) was below the detection limit (<0.01%) showing no BC decomposition products in water leached from the soil.

We conclude that applying ^{14}C labeled BC opens new ways for very sensitive tracing of BC transformation products in released CO_2, microbial biomass, DOC, and SOM pools with various properties.

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1. Introduction

Black carbon (BC) 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; Dickens et al., 2004). The interest in BC 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 time periods, because its microbial decomposition and chemical transformation is apparently very slow. This conclusion of chemical and biological inertness of BC is mainly based (references in Forbes et al., 2006) on its: 1) high resistance to a range of chemical oxidants, 2) preservation for long periods in geological record, and 3) existence at soil depths where the residence time exceeds 1000 years.

Various descriptive methods of BC identification (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., 2005a,b) have been developed. Comparison of the BC identification and quantification approaches showed that the results were strongly dependent on the methods used (Schmidt et al., 2001; Forbes et al., 2006; Hammes et al., 2007). Improved descriptive methods were used to estimate burial periods, which, in turn, reveal fire history (Ballentine et al., 1998), allow BC detection in soils and sediments as well as the evaluation of BC sources (Glaser and Knorr, 2008).

In contrast to the descriptive methods there are only few studies describing BC transformation processes in soils and there is a lack of papers estimating the rates of BC transformations (Brodowski et al., 2005a; Glaser et al., 2000). Based on scanning electron microscopy (SEM) coupled to an energy-dispersive X-ray spectrometer (EDX), Brodowski et al. (2005a) showed that BC is slowly oxidized and may be bound to mineral particles. However, this approach is not applicable to the oxidation time periods necessary to evaluate BC decomposition or transformation rates. Comparing historical BC samples and newly produced BC, Cheng et al. (2008a) showed substantial oxidation of BC during 130 years and related the oxidation intensity to the mean annual temperature.

The lack of studies estimating process rates is connected with BC inertness 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 BC in soils. Direct evidence of long MRT is based mainly on $\Delta^{14}C$ measurements of BC in soils (Schmidt et al., 2002; Gavin et al., 2003) and marine sediments (Masiello and Druffel, 1998). As the microbial activity in marine sediments is extremely low, the MRT observed there cannot be transferred to BC in soils. No studies estimating BC decomposition rates in soils are available. This is because the BC content changes in soils are too small for any practical experimental period (months to 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 BC decomposition rates because of the much higher contributions of soil organic matter (SOM) and plant residues mineralization to the CO_2 compared to BC. To solve this problem, Hamer et al. (2004) incubated BC with carbon-free sand and estimated BC decomposition rates based on total CO_2 efflux from the BC–sand mixture. Depending on the BC origin, between 0.3 and 0.8% of the added BC were mineralized in sand during 60 days at 20 °C. Hamer et al. (2004) also showed that an additional, easily available C source (glucose) may prime microbial BC decomposition by up to 1.2% in the same period. Such an effect of glucose on BC decomposition led us to assume that in soils the decomposition rates of BC could be different from those in pure sand.

To distinguish between the CO_2 efflux from BC and from glucose decomposition, as well as to estimate interactive effects on the decomposition, uniformly ^{14}C labeled glucose was used (Hamer

et al., 2004) and the contribution of BC to total CO_2 efflux was estimated as the difference. Whereas this labeling approach is very useful for estimating the contribution of CO_2 sources with comparable decomposition rates (Kuzyakov et al., 2007; Schneckenberger et al., 2008), it may be strongly biased when the contribution of the unlabeled source is <10%. Considering analytical and experimental errors of 3%, for example, this approach yields the estimation error of more than ±100% when the contribution of the unlabeled source is <3% of total CO_2 efflux. This uncertainty strongly increases when the contribution of the unlabeled source decreases (BC in the study of Hamer et al., 2004) and when experimental errors increase.

Decomposition rates of organics in soil depend both on the substance properties and on the accessibility to microorganisms and their enzymes (Ekschmitt et al., 2008; Lützow et al., 2006). Brodowski et al. (2006) suggested that the BC stability in soils does not solely reflect its refractory structure, but also its poor accessibility when physically enveloped by soil particles. The highest BC contents were found in the fraction of small microaggregates (<53 µm) and the smallest BC contents in the large macroaggregate fractions (>2 mm). They therefore concluded that stabilization within microaggregates plays a significant role in additionally reducing BC decomposition rates (Brodowski et al., 2006). However, these results of preferential BC stabilization in microaggregates contradict the turnover times of these aggregates, which were estimated to average 88 days (De Gryze et al., 2006, based on rare-earth oxides). Accordingly, if the BC decomposition rates lie within hundreds or even thousands of years (Preston and Schmidt, 2006), then an additional retardation in the range of hundreds of days by microaggregate turnover will not significantly affect BC decomposition. It remains unclear, whether the occlusion of BC in aggregates really prolongs BC decomposition. Therefore, our experimental layout included intensive mixing of soil to simulate tillage and to evaluate possible effects of aggregate destruction on acceleration of BC decomposition.

Considering the absence of BC decomposition studies in soils, as well as the possible errors involved in estimating decomposition rates based on BC-derived CO_2 efflux (calculated by the difference method), we used an alternative approach. We produced ^{14}C labeled BC by charring shoot litter of *L. perenne* uniformly labeled with ^{14}C, mixed the BC with soil or nearly C_{org}-free loess, and incubated for 3.2 years. Adding glucose and intensive mixing should simulate the possible stimulation of BC decomposition (i.e. in rhizosphere conditions) or the destruction of soil aggregates, respectively.

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 field experimental station Karlshof of Hohenheim University, Stuttgart, Germany) (Haploxeralf). The soil originated from loess; it contains no $CaCO_3$ and has the following characteristics: pH 6.0, C_{org} 1.2%, N_t 0.13%, clay 23%, silt 73%, and sand 4.4%. The soil was air-dried, sieved on a 2-mm screen, and 45 g dry soil were filled in the incubation jars.

Loess samples for the experiment were taken from an open-cast mine at Nussloch, SW Germany (49.19°N, 8.43°E, 217 m asl) (Bente and Löscher, 1987). A set of thermoluminescence and two radiocarbon dates suggest that most of the loess–palaeosol sequence formed during the last glacial–interglacial cycle (Zöller et al., 1988; Hatté et al., 1998). The loess sample for the experiment was taken from 15 m depth, presumably from a middle to lower pleniglacial section of the loess–palaeosol sequence. The sample represented a loose, sandy–loamy material, light yellowish-gray in color; it

contained 27% $CaCO_3$. 44.1 g of loess (air-dried and sieved on a 2-mm screen) 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 C_{org} g^{-1}. This loess–soil mixture is termed here as loess.

2.2. ^{14}C labeled pyrogenic carbon

^{14}C labeled pyrogenic carbon was produced from shoot litter of *L. perenne* that was uniformly labeled with ^{14}C. The litter of *L. perenne* represented the 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). Separate experiments at various temperatures (from 200 to 400 °C) and durations (from 3 to 13 h) of charring were conducted to find optimal conditions for charred carbon production. This was necessary to minimize losses of ^{14}C labeled plant material during the charring. In these charring experiments, unlabeled shoot litter of *L. perenne* was used, and the optimal conditions were evaluated based on mass and C losses during the charring. According to C losses by charring (see Section 3), the following conditions for production of ^{14}C labeled charred C were chosen: slow heating during 4.5 h from 20 to 400 °C following by 13 h at 400 °C. Thereafter, the muffle furnace was switched off and left open to cool to room temperature. The produced BC was pitch black. It was mixed again and 108 mg BC 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% of the C_{org} in soil or nearly 200% of C_{org} in loess and a total ^{14}C activity of 1.44 kBq per jar. Soil or loess was thoroughly mixed with added BC before incubation.

2.3. Experimental layout and incubation conditions

To investigate BC decomposition, two substrates were used: soil originated from loess and loess with a very small amount of organic C. Soil or loess without BC addition was used as a control for the total CO_2 efflux. To check the effect of easily available substrate on BC decomposition, unlabeled glucose was added five times to the soil or to loess jars containing ^{14}C labeled BC. Glucose was added to the same jars on days 26, 132, 280, 587 and 1089 at an amount of 2.16 mg glucose per g soil (except d 132). At the second addition (d 132), 48 μg g^{-1} were added. This resulted in the addition of 0.86 mg C g^{-1} soil or loess for days 26, 280, 587 and 1089, and of 19 μg g^{-1} for day 132. To check the effect of mechanical disturbance, soil or loess of another treatment was carefully mixed in the jars for 1 min on days 26, 132, 280, 587 and 1089. To distinguish fast processes at the start of incubation and directly after each glucose addition or mixing, the NaOH was exchanged much more frequently than in periods without treatments.

Before the first glucose addition or mixing (first 25 days), the data were collected with 16 replications for BC treatments and with 4 replications for the control without BC. After d 26, all treatments were conducted with 4 replications. The BC treatments without glucose addition and without disturbance were conducted with 8 replications.

The samples of 45 g soil or loess material with or without BC were incubated in 250-mL Schott jars for 1181 days at 20 °C and 70% of WHC. To obtain 70% of WHC, 9.45 and 8.82 mL distilled water were added 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 1181-d period, the NaOH trap was exchanged 44 times. At day 624 (1.7 years), incorporation of ^{14}C from BC into microbial biomass and DOC was determined by the fumigation–extraction method.

2.4. Sample analysis

The ^{14}C activity of CO_2 trapped in a NaOH solution was measured in 2 mL aliquots with 4 mL of the scintillation cocktail for hydrophilic solutions (EcoPlus, Roth Company) after the decay of chemiluminescence. The ^{14}C counting efficiency was about $89 \pm 1\%$ and the ^{14}C-activity measurement error did not exceed 2%. The absolute ^{14}C activity was standardized by adding NaOH solution as a quencher to the scintillation cocktail and using a two-channel ratio method of extended standard (tSIE). 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 for each sample.

The total content of $C–CO_2$ collected in the NaOH solution was measured by titration of 1 mL aliquot with 0.2 M HCl against phenolphthalein after addition of 0.5 M $BaCl_2$ solution (Zibilske, 1994). Total C and N content of the soil, loess, *Lolium* shoots and charred C was measured using a LECO elementary analyzer.

Soil microbial biomass was determined by the chloroform fumigation–extraction method (modified after Vance et al. (1987)). One gram of soil or loess was extracted with 4 ml of 0.05 M K_2SO_4 solution. Another 1 g of soil was first fumigated with chloroform for 24 h and then extracted in the same way. The K_2SO_4 and soil mixtures were shaken for 1 h at 200 rpm, centrifuged at 3000 rpm for 10 min, and then filtered through a ceramic vacuum filter. The extracts were frozen until analyses for total C concentrations using a 2100 TOC/TIC analyzer (Analytic Jena, Germany). Microbial biomass C concentrations were calculated from these results by using a kEC value of 0.45 for C and ^{14}C (Wu et al., 1990) and were presented as percentage from 1 g of dry soil. The soil water content was determined in another 1 g of soil that was dried at 105 °C.

2.5. Calculations and statistics

All results for cumulative CO_2 were recalculated for mg C g^{-1} soil or loess. The CO_2 efflux rates are presented as mg C g^{-1} d^{-1}. The decomposition of BC is presented as percentage from the ^{14}C input activity (100%).

To calculate the significance of differences between the treatments, repeated measures ANOVA was used. The significance of differences is presented as p (error probability) value. The standard error of means is presented in figures.

3. Results

3.1. Production of ^{14}C labeled Black Carbon from Lolium residues

To optimize BC production from ^{14}C labeled plant residues, the unlabeled residues of *Lolium* shoots were charred for different periods at increasing temperatures (Fig. 1). The temperature range varied from 200 to 400 °C and the charring duration between 3 and 13 h. We did not go further increasing the temperature, since BC from vegetation fire is generally produced at temperatures lower than 450 °C (Chandler et al., 1983). Within these temperature and duration ranges, both parameters almost linearly decreased the mass of residues remaining after the charring. For the production of BC from ^{14}C labeled *Lolium* residues, 13 h charring at 400 °C were chosen. After this charring regime the initial residue mass decreased up to 33% of the initial value and the C content of the produced BC was 56% of the initial weight. This corresponds to the C content in BC produced from various grasses (Forbes et al., 2006). Thus, 55% of the initial C content of the plant residues was lost
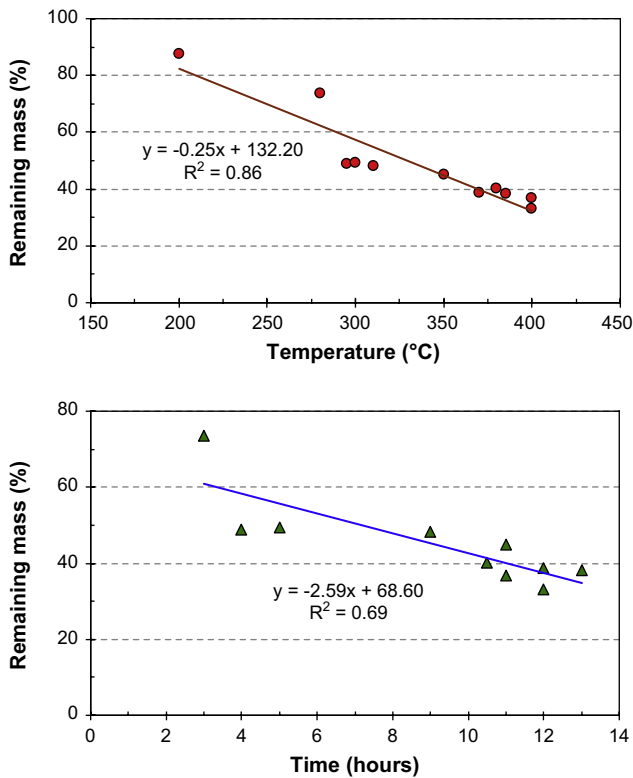
Fig. 1. Changes of charred mass of *Lolium perenne* (L) shoots as function of temperature (top) and duration (bottom) of charring.

during the charring. The produced BC was pitch black and consisted of very fine particles.

3.2. Total CO_2 efflux from soil and loess with and without BC

The initial CO_2 efflux rates from the soil were 41.8 µg CO_2–C g^{-1} d^{-1}. This was about 30 times higher than the initial CO_2 efflux rates from loess (data not shown). However, after two weeks of incubation, the CO_2 efflux rates from the soil strongly decreased and those from loess increased. This yielded a soil to loess ratio of 2–2.5. After one year of incubation, the CO_2 efflux rates from the soil were 1.63 ± 0.25 and 1.75 ± 0.17 µg CO_2–C g^{-1} d^{-1} with and without BC, respectively. Note that about half of the individual measurements of CO_2 efflux rates were not significantly different from the control without BC. The respective rates for loess without and with BC were 1.56 ± 0.45 and 1.08 ± 0.10 µg CO_2–C g^{-1} d^{-1}.

Adding BC did not change the total CO_2 efflux rates from soil and from loess within 2 months of incubation. Thereafter, BC had contrasting effects on CO_2 efflux from soil and from loess. BC slightly increased CO_2 efflux rates from soil for about 0.6 µg CO_2–C g^{-1} d^{-1} and significantly decreased it from loess for 0.2–1.5 µg CO_2–C g^{-1} d^{-1} (Fig. 2). Intensive mixture of soil or loess with BC did not significantly change the total CO_2 efflux from soil or from loess (Fig. 2).

3.3. Decomposition of BC

The decomposition of BC was estimated based on the $^{14}CO_2$ efflux (Fig. 3) because the changes in the total CO_2 efflux after BC addition were too small (Fig. 2) to allow relevant conclusions. The $^{14}CO_2$ efflux produced by BC mineralization showed a very slow decomposition. Less than 4.5% of the ^{14}C added as BC was released as $^{14}CO_2$ during 3 years (Fig. 3, top). The BC mineralization rates

(Fig. 3, bottom) strongly decreased during the incubation: in the first month the BC mineralization amounted to 0.016 and 0.024% d^{-1} for soil and loess, respectively. After one year the mean BC mineralization decreased by more than one order of magnitude and amounted to 0.0012 and 0.0016% d^{-1} for soil and loess, respectively. At the end of the study (3.2 years) the BC decomposition rate was similar for soil and loess and amounted to 0.00136% d^{-1}. There was no significant effect of soil or loess on BC mineralization at individual sampling dates. Based on the curve of BC mineralization rates and cumulative $^{14}CO_2$ evolved (Fig. 3), we conclude that the BC mineralization will further decrease.

3.4. Priming effect of glucose and mechanical disturbance effect on BC decomposition

Five additions of an easily available external energy source (glucose) as well as five intensive mixing events of the soil or loess accelerated BC mineralization by several times (Fig. 4). However, the accelerating effect of both treatments ended within a few weeks and the $^{14}CO_2$ efflux rate did not differ from the control. For all five treatments the effect of glucose was much higher than that of the mixing (Fig. 4). Except for the fourth glucose addition, the effect of glucose was strongly pronounced on loess, which had a much lower C_{org} content compared to the soil.

The duration of the effect of glucose or mixing never extended beyond one month after the treatment. Therefore, we averaged the rates of $^{14}CO_2$ efflux within one week and one month after each treatment and compared the $^{14}CO_2$ efflux rates with the control (Fig. 5). All the effects averaged for one week (Fig. 5, left) were positive and 3–5 times higher than the effects averaged for one month (Fig. 5, right). Maximal values of the priming effect averaged for one month after glucose addition in the soil were about 160% of the control. The corresponding values for loess varied between 20 and 144% (Fig. 5, right). Note, however, that these priming effect values are calculated as $^{14}CO_2$ efflux rates averaged for one month after the respective treatment. The priming effect shortly after the treatment was much higher. Accordingly, if the priming effect was calculated as averages during one week after treatment, then the effects were always positive and reached up to 620% of the control (Fig. 5, left bottom). The priming effect after glucose addition was always strongly positive and was typically higher than the effect of mixing.

3.5. Incorporation of BC into microbial biomass

Microbial biomass measured by the fumigation–extraction approach 624 d after the start of incubation was higher in the soil (740 µg g^{-1}) than in the loess (418 µg g^{-1}) (Fig. 6, left). Even though more than 96% of the added BC remained in the soil or loess after 624 d, the incorporation of ^{14}C into microbial biomass was 2.6 and 1.5% of ^{14}C input for soil and loess, respectively (Fig. 6, middle). The relative incorporation of BC into microbial biomass was similar in the soil and loess (Fig. 6, right).

It is necessary to mention that chloroform can contribute to partial dissolution of BC remaining in partly decomposed plant residues. Moreover, the application of the extraction coefficient kEC of 0.45 commonly used for microbial biomass estimations, is very questionable for BC products and may additionally increase the estimated incorporation of ^{14}C from BC into microbial biomass. Therefore, the values obtained by the fumigation–extraction approach for incorporation of ^{14}C from BC into microbial biomass reflect the maximal possible values.

No ^{14}C from BC was measured in dissolved organic carbon (DOC) extracted by K_2SO_4 solution from soil or loess.
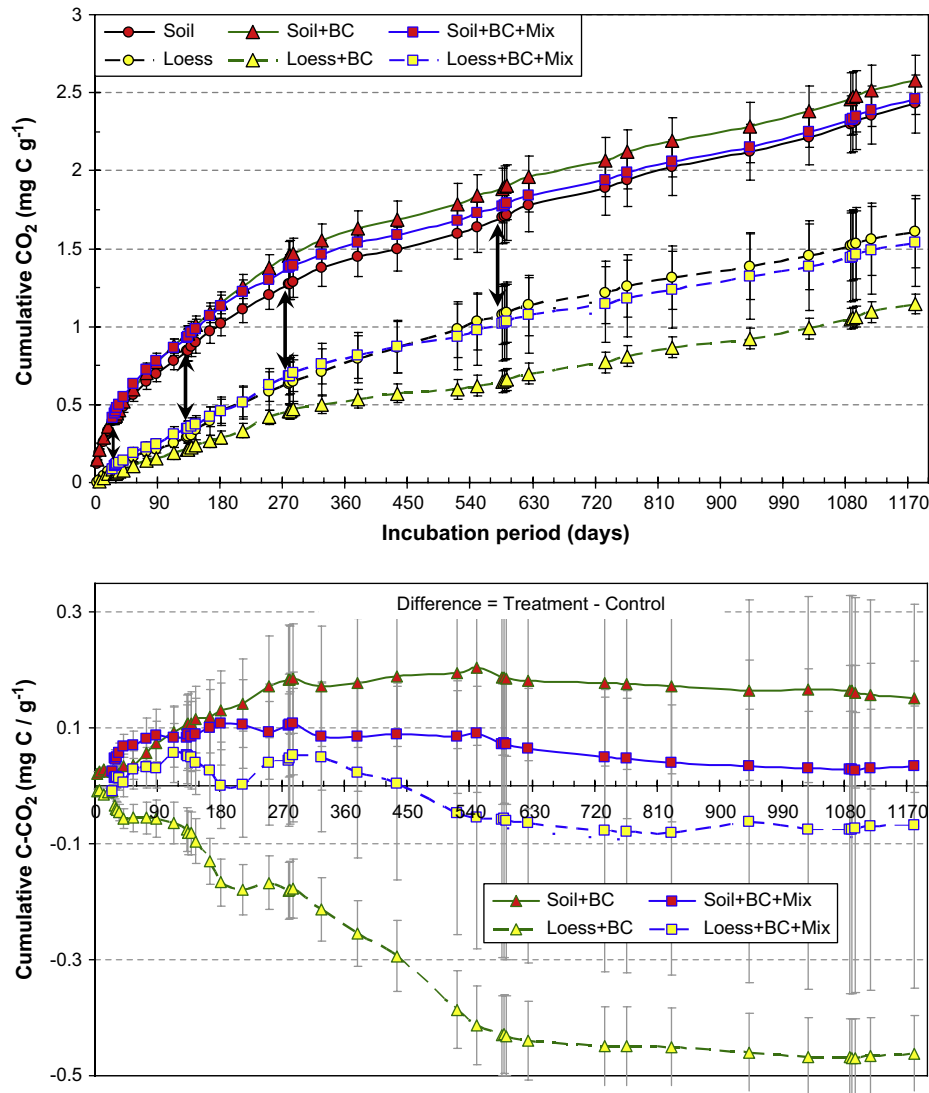
Fig. 2. Top: cumulative CO_2 efflux from soil and loess as affected by black carbon (BC) addition and intensive mixing. Vertical black arrows show the time of five intensive mixings. Bottom: changes of CO_2 efflux from soil and loess with BC compared to the respective control treatments without BC addition. Error bars show standard errors ($n = 8$ for BC and 4 for control and mixing treatments). The treatments with glucose are not shown here because of intensive CO_2 production directly after glucose addition.

4. Discussion

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

Our study is the first one to estimate BC decomposition in soil directly. Transformation of BC in soils (Preston and Schmidt, 2006) and sediments is a very slow process. This causes failure of the most common methods based on changes of total BC–C in the soil or in CO_2 efflux because the contribution of BC is very small against the background of high CO_2 fluxes from other sources, i.e. SOM, dead plant residues, DOC etc. We produced BC from ^{14}C labeled plant residues to distinguish the C released from BC to CO_2 or incorporated into microbial biomass from all other unlabeled C sources. Although the specific ^{14}C activity of the produced BC was low (13.3 Bq mg^{-1}), it was sufficient to estimate BC decomposition rates of 10^{-5} d^{-1} ($=10^{-3}$% d^{-1}). Such a high sensitivity allowed a precise estimation of BC decomposition against the background of high CO_2 fluxes from other sources (Fig. 3), even though the direct contribution of the BC to the CO_2 efflux was not measurable (Fig. 2).

Note that the application of BC with a shifted ^{13}C signature has already been tested to evaluate the origin of BC. By using positive and negative ^{13}C labels at natural abundance levels, Glaser and Knorr (2008) found that up to 25% of BC in soils may be produced *in situ*, without fire or charring (i.e. is of biological origin). The isotopic signature of BC has also been used to reconstruct the predominance of C_3 and C_4 vegetation (reviewed in Glaser, 2005). However, all known studies linking BC with ^{13}C signatures were done at the ^{13}C natural abundance level. Considering the very slow BC decomposition rate, the ^{13}C natural abundance is inappropriate for estimating BC decomposition rates.

BC decomposition rates can probably be estimated not only using ^{14}C, as was done in this study, but also using BC produced from ^{13}C labeled plant residues. However, this would require a very high enrichment of ^{13}C in the BC and therefore in the initial plant material source (>50%) to obtain similar sensitivity. This is unrealistic because CO_2 with such a high ^{13}C enrichment would need to be provided to the plants throughout the growth period (continuous labeling). In contrast to ^{13}C, the production of BC with a much higher specific ^{14}C activity is a comparatively simple issue; it may further increase the sensitivity of the approach by 3–5 orders of magnitude.

Application of ^{14}C labeled BC opens a new ways to trace not only the BC itself, but also its transformation products. Thus, the C from
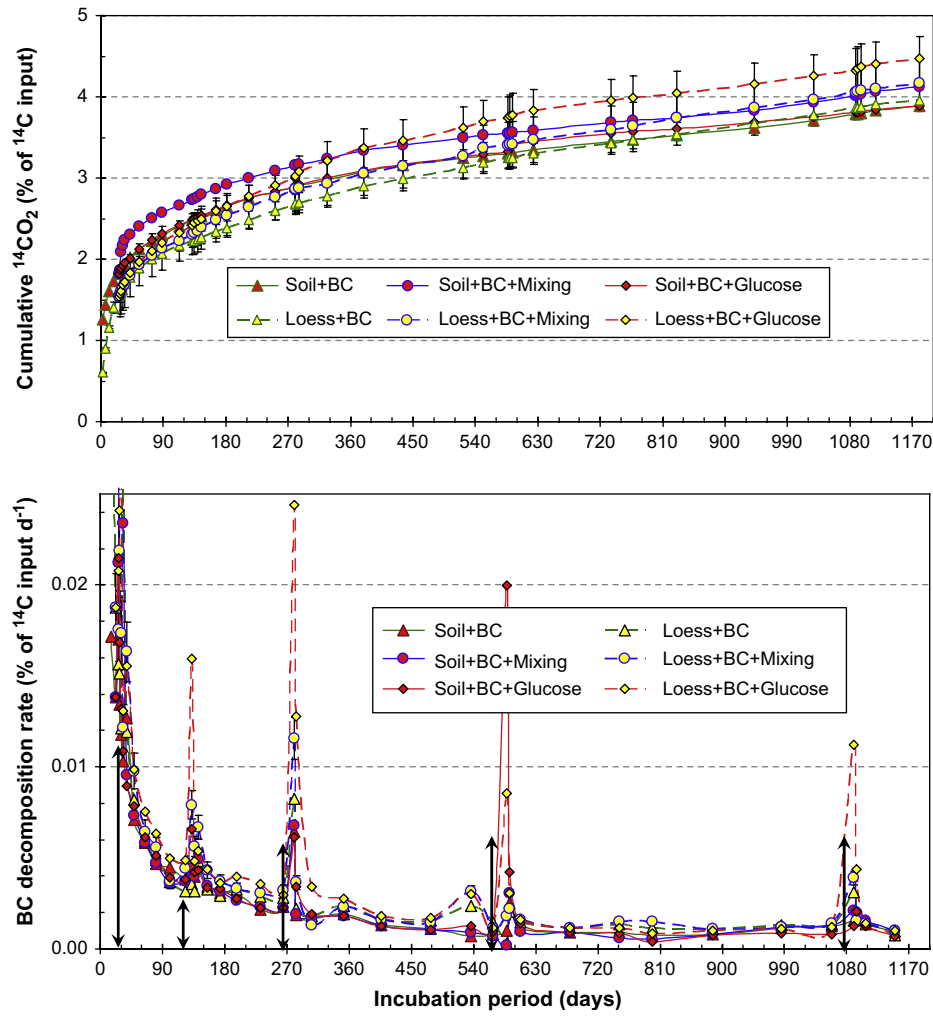
Fig. 3. Black Carbon (BC) mineralization (±SE) in soil and loess as affected by 4 glucose additions or intensive mixings. Top: cumulative $^{14}CO_2$ efflux; bottom: BC mineralization rates. Vertical black arrows show the time of five glucose additions or intensive mixings.

BC can be traced in microorganisms, and DOC (as in this study), can be coupled with particle size and density fractionation. If BC is further stabilized by physical or chemical mechanism, then ^{14}C labeling allows it direct analysis in individual SOM pools and aggregates.

4.2. Decomposition of BC and its MRT in soil

As expected here and as assumed in other studies, the BC decomposition rate was very slow. These rates may be separated into two periods. During the first two to three months, the BC
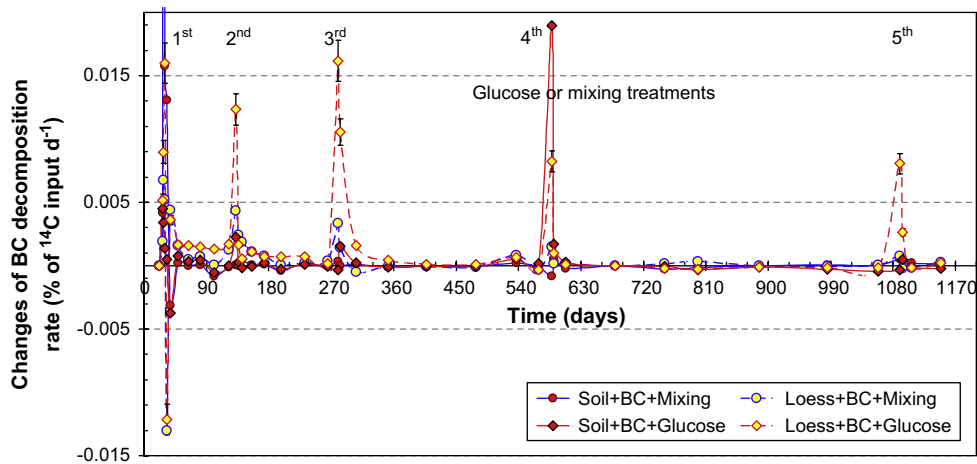

Fig. 4. Effect of glucose additions or intensive mixings on Black Carbon (BC) mineralization in soil and loess. Note the different BC mineralization rates (±SE) with glucose additions or intensive mixings versus the control without glucose or mixing.
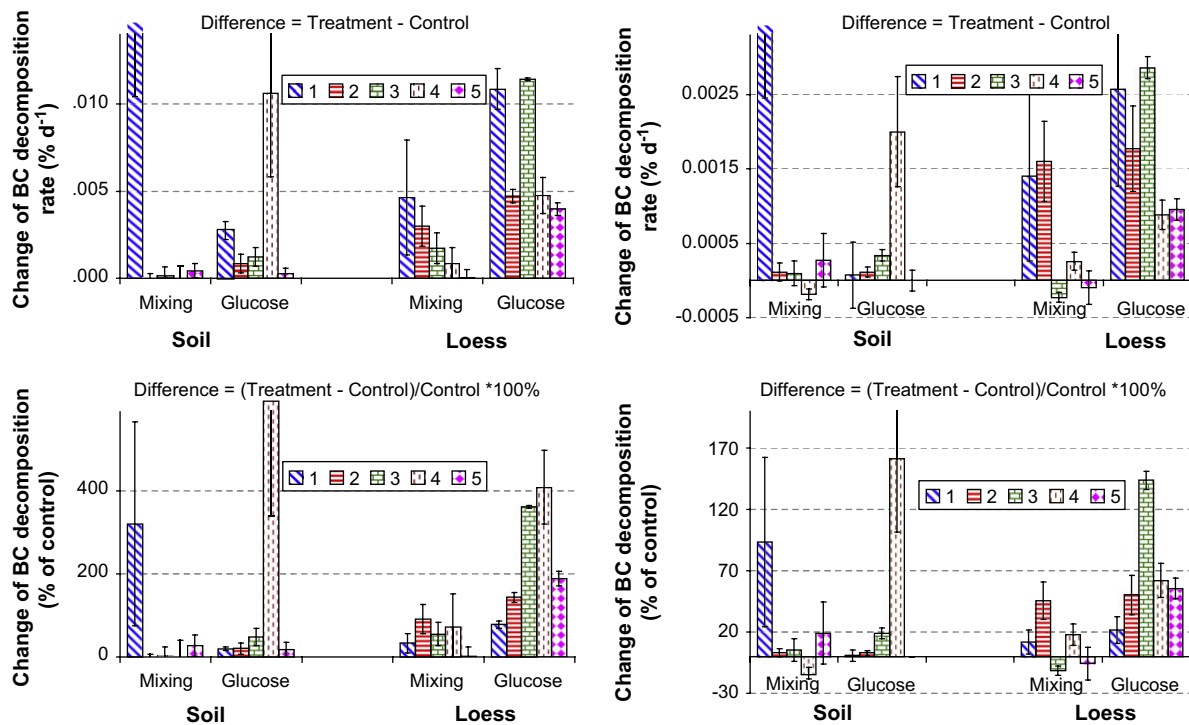
Fig. 5. Priming effect of glucose and effect of mechanical mixing on Black Carbon (BC) mineralization ($\pm$SE) in soil and loess averaged for one week (left) and for one month (right) after the treatment. Top: changes of BC decomposition rates (% of BC input d^{-1}); bottom: changes of BC decomposition rates related to the decomposition rates in the control (% of control). The numbers correspond to the number of the treatment: 1 (d 262), 2 (d 132), 3 (d 280) and 4 (d 587). Note different Y scales for rates averaged for one week (left) and one month (right).

decomposition was comparatively high (up to 0.05–$0.15\% \, d^{-1}$). After two months, 2.10 ± 0.07 and $1.84 \pm 0.04\%$ of added BC were decomposed in soil and loess, respectively. These values are 2–3 times higher than the decomposition of rye and maize charred residues during 60 days under similar conditions (0.72–0.78% of BC–C input) (but in C-free sand instead of soil; Hamer et al., 2004). The faster decomposition is the result of higher microbial and enzymatic activities in soil versus sand.

After the initial fast decomposition phase lasting two to three months, the BC decomposition decreased, remaining at 0.0013–$0.0015\% \, d^{-1}$ during the second and third years. As this rate slowly but continuously decreased during the 3.2 investigation years, we expect it to further decrease. Such a decrease of the decomposition rate reflects not only potential protection of some BC particles within soil aggregates, but also continuous preferential utilization of BC compounds (i.e. very small particles, strongly oxidized parts), which are more degradable than others. Cheng et al. (2008a,b)

showed that the proportion of a more labile fraction of BC progressively decrease leading to increase of BC half-life during the incubation. BC composition is not uniform, and it consists of a broad range of substances of different C condensation, aromatics, oxygen content, etc. (Preston and Schmidt, 2006; Hammes et al., 2007). Therefore, preferable utilization of some compounds during initial decomposition will slow decomposition in the following stages.

Brodowski et al. (2005a) showed that oxidized BC particles may be bound to soil minerals thus decreasing BC decomposition. However, it is questionable whether such binding is a significant process during the three years of our incubation experiment.

Based on the BC decomposition rates measured under controlled conditions ($20\,^{\circ}C$, 70% aWHC, loamy texture), we can roughly estimate the BC decomposition and its residence time under field conditions. According to the Biological Active Time approach (Franko et al., 1997) proven by many controlled-condition
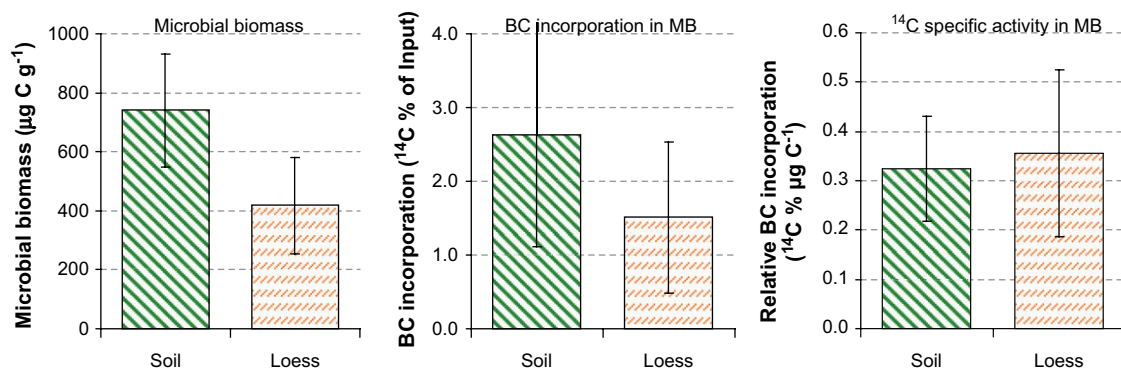

Fig. 6. Incorporation of ^{14}C from Black Carbon (BC) into microbial biomass (MB) after 624 days of incubation in soil and loess. Left: microbial biomass content; middle: ^{14}C from BC into microbial biomass; right: relative incorporation of ^{14}C into microbial biomass. Error bars show standard errors ($n = 4$).

and field decomposition studies, the biological activity of a loamy soil in the field (MAT = 7 °C, MAP = 600–700 mm) is about 10% of that under optimal conditions. Accordingly, the BC decomposition rates we measured here under optimal conditions are about one order higher than in the field. As the mean residence time (MRT) is a reciprocal of the decomposition rates (Kuzyakov, 2002), the MRT of BC under optimal conditions will be about 200 years. Assuming the 10% biological activity value under field conditions, the MRT of BC will be about 2000 years. This MRT corresponds to the half-life of BC in soil of about 1400 years. This MRT is in the range of residence time of BC in European Chernozems (1160–5040 years) estimated by Schmidt et al. (2002). It is shorter than the MRT of BC in ocean sediments (2400–13900 years) estimated by Masiello and Druffel (1998). Both results are based on radiocarbon dating.

Importantly, the sources of BC in soil may vary and are not always connected with fires. As shown by Glaser and Knorr (2008), substances having a chemical structure similar to BC may be produced by fungi, i.e. *Aspergillius niger*. The BC decomposition rates calculated in this study are therefore relevant only for pyrogenic BC and cannot be directly transferred to BC of biological origin.

We are aware that extrapolating BC decomposition rates from an incubation study to the field is speculative. Despite the lower Biological Active Time in the field versus the optimal conditions study (Franko et al., 1997), many other factors may stimulate BC decomposition in the field. These are 1) freezing/thawing cycles, which may disperse BC particles and increase their active surface, 2) drying/wetting, which may increase the local concentration of enzymes at the BC surface, 3) rhizosphere processes close to the living roots, mainly connected with stimulation of microbial activity and increasing C turnover, and 4) soil mixing by animals and aggregate destruction by living roots. We assumed the last two processes to be more important than the first two and considered them in this incubation study.

4.3. Priming effect of glucose on BC decomposition

Adding glucose to the soil may partly reflect the increase of microbial activity in the rhizosphere, because sugars are the main components of root exudates and other rhizodeposites (Kraffczyk et al., 1984; Merbach et al., 1999). Such addition of glucose to the soil and loess with BC stimulated the BC decomposition by up to 6 times (Figs. 3 bottom, 4 and 5). However, this stimulation was only for short period (one week). For the whole incubation period this increase was marginal (Fig. 3, top).

As glucose does not directly affect BC availability but is an important C and energy source for microorganisms, the stimulated BC decomposition is clear evidence for cometabolic decomposition (Hamer et al., 2004). This means that microorganisms are not dependent on BC utilization as a C or energy source, but microbial enzymes produced for decomposition of other substrates (here glucose and its metabolites) contribute to BC decomposition (Kuzyakov et al., 2000; Hamer et al., 2004). This is also confirmed by higher effect of glucose on BC decomposition in loess (having very low organic C content) compared to the soil (Fig. 5). Considering cometabolic BC decomposition, we disagree with Brodowski et al. (2005a) that microorganisms decompose BC to get access to nitrogen sources. An indirect evidence of cometabolic decomposition is the strong drop in the stimulation of BC decomposition one week after glucose addition, when the glucose was decomposed or completely transformed to microbial metabolites. After one month, when the decomposition of glucose metabolites was completed, the BC decomposition did not significantly differ from the control without glucose (Figs. 3 bottom and 4).

4.4. Effect of mechanical disturbance on BC decomposition

Mechanical disturbance, simulated in the study by intensive soil mixing, also promoted BC decomposition (Figs. 3–5). We assume here that the stimulation of BC decomposition by soil mixing involved the destruction of soil aggregates in a two-fold manner. The first one is direct: some of the BC particles enclosed in the aggregates may not directly be accessible for microorganisms or their enzymes (Brodowski et al., 2006), and mixing brings the BC particles to the aggregate surface. The second is indirect: newly emerged surfaces after mixing increase the availability of C sources for microorganisms. These new C sources have a similar effect as the glucose addition described above. This was confirmed by the total CO_2 efflux from the soil after mixing: values increased 1.5–7 times as compared to the control without mixing (data not shown). Since the total CO_2 efflux from the soil increased by a factor of 1.5–7 and BC decomposition only by a factor of 0.5–2 (Fig. 5 bottom), we assume that the main effect of mixing on BC decomposition was connected with the second reason – increased substrate availability induced cometabolic BC decomposition. The effect of mechanical disturbance is therefore mainly indirect – by increasing of availability of other C sources for microorganisms. This, in turn, stimulates microorganisms for additional BC decomposition.

4.5. Effect of Black Carbon on native soil organic matter decomposition

Recently, strong stimulation of forest humus decomposition in the presence of BC was reported based on the mass loss from litter bags (Wardle et al., 2008). This stimulating effect was explained by preservation of some nutrients from leaching in BC, subsequent increase of microbial activity in the BC treatments induced humus decomposition compared to the treatment with humus only. Based on this result it was speculated that BC input in boreal soils may stimulate native soil C decomposition and lead to decrease of C sequestration (Wardle et al., 2008). In our incubation study with clear separation of the C sources in CO_2 based on ^{14}C, no significant effects of BC on native soil C decomposition were found for Haplic Luvisol (Fig. 2b). Additionally, we found a decrease of CO_2 production from loess in the presence of BC (Fig. 2b). We explain this decrease by sorption of nutrients and available organic C on BC and strong additional limitation of microbial activity in loess with very low levels of all nutrients and available organic C. These results concur with decrease of SOC mineralization and increase of its half-life in the presence of 130 years old BC (Cheng et al., 2008b). Therefore, the stimulation of humus decomposition by BC presented by Wardle et al. (2008) needs thorough check for various ecosystems including calculation of C budget: leached C and released as CO_2 as well as identification of C sources by ^{14}C or ^{13}C (Lehmann and Sohi, 2008).

4.6. Incorporation of BC into microbial biomass and dissolved organic carbon

This study appears to be the first one showing direct incorporation of C from BC into microbial biomass. The amount of BC–C incorporated into microbial biomass shows which portion of the substrate is being used by microorganisms at the time of sampling. It indirectly reflects microbial availability of the substrate. Only between 1.5 and 2.6% of the remaining 96% BC were incorporated into microbial biomass after 624 incubation days. This shows an extremely low microbial availability of BC and indirectly confirms our statement that BC will be decomposed mainly by cometabolism and is of negligible importance as a C source for microorganisms.

However, the incorporation of minimal amounts of BC into microbial biomass should be interpreted with caution: fumigation

by $CHCl_3$ may solubilize not only actual microbial cells but also non-living soil organic matter. Badalucco et al. (1990) showed that a very low amount (0.2–0.4% of total C content) of non-living soil organic matter was dissolved by $CHCl_3$ fumigation. This means that between 10 and 25% of BC incorporated into MB may reflect methodological shortcomings of $CHCl_3$ fumigation. At the same time, we can be certain that more than 75% of the BC released after $CHCl_3$ fumigation was really allocated in microbial cells.

We found no ^{14}C from BC in DOC. This contrasts with the result that atmospheric BC became soluble after oxidation (Decesari et al., 2002). Those authors, however, evaluated water-soluble organic carbon after soot oxidation in an ozone atmosphere. Therefore, oxidation conditions much stronger than those present in soil may produce water-soluble substances.

The sensitivity of our approach based on specific ^{14}C activity of BC and its analysis by common liquid scintillation counting of one sample over 1 h is about 0.01% of initial BC content. Accordingly, if the amount of BC that became soluble is less than 0.01% of input, we cannot measure it. This sensitivity may be increased by 3–5 orders of magnitude if the specific ^{14}C activity of the BC is higher.

5. Conclusions

Direct estimation of BC decomposition in soil under optimal conditions showed very slow rates of about $1.36\ 10^{-5}\ d^{-1}$, corresponding to a decomposition of about 0.5% BC per year. Considering the much slower decomposition under field conditions, we estimated the mean residence time of BC in soils of temperate climates to be about 2000 years. Glucose amendment as a C and energy substrate for microorganisms strongly stimulated BC decomposition. However, this stimulation was strongly pronounced for the first week and lasted maximum one month. Both results indicated that BC decomposition mainly involved cometabolism. Intensive soil mixing had a smaller and shorter stimulation effect. We explain this mainly by the release of additional substrate on new aggregate surfaces. The incorporation of BC in microorganisms varied between 1.5 and 2.6% of BC input, confirming our hypothesis on cometabolic BC decomposition by microorganisms. As the amount of BC in dissolved organic carbon was below the detection limit, no contamination of ground water by BC decomposition products can be expected.

We conclude that applying ^{14}C labeled BC to investigate BC decomposition and utilization by microorganisms (and probably other transformations as well) opens new possibilities to investigate very slow processes because of the high sensitivity of ^{14}C analytics. This approach also allows calculation of the C budget of applied BC and identification of BC products in SOM fractions and microbial pools. ^{14}C labeled BC may also be used for evaluation and optimization of BC analytics in soils and sediments.

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