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The role of lignite in the carbon cycle of lignite-containing mine soils: evidence from carbon mineralisation and humic acid extractions

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Abstract

In many mine soils, lignite carbon is present as a potential carbon source for micro-organisms. Although the establishment of an active microbial community in reclaimed mine soils was recognised to be of great importance to reclamation success, the organic substrates available for micro-organisms have never been characterised in terms of bioavailability. The objective of this study was to examine if lignite in mine soils is decomposed and transformed during biodegradation. A chronosequence of lignite-rich mine substrates consisting of lignite-containing overburden material (parent substrate for soil development), a 14 years old mine soil and a 37 years old mine soil, both rich in lignite in addition to recent carbon were sampled and incubated for 16 months. Carbon mineralisation was monitored and the soil-respired CO_2 subjected to ^{14}C activity measurements. Additionally the ^{14}C activity of humic acids extracted from the soils was determined. With the ^{14}C activity data, lignite carbon contribution was estimated. These results show that lignite was decomposed in the lignite-containing parent substrate as well as in the 14 and 37 years old mine soil over the whole incubation period, the average decay rate of lignite being 0.025 and 0.007 g lignite C kg^{-1} C $year^{-1}$. Lignite carbon was part of the humic acid fraction, indicating that lignite in the soil is oxidized during biodegradation A higher portion of lignite can be extracted as humic acids with increasing soil development. Thus, lignite in soil can be mineralised as well as humified and must be considered in the soil carbon cycle.

1. Introduction

The reclamation success of lignite mine sites depends on the establishment of functioning biochemical cycles (Tate, 1985). For the establishment of the carbon cycle, the formation of soil humus is particularly important (Leiros et al., 1993). Intimately linked with this process, and a most sensitive indicator for the reclamation success, is the microbial biomass and its activity (Insam and Domsch, 1988; Gil-Sotres et al., 1992). The accumulation of recent organic matter (OM) can be detected in the upper few centimetres of very young mine soils (Roberts et al., 1988; Leiros et al., 1993; Malik and Scullion, 1998): apart from this, little is known about the biochemical evolution of these soils (Gonzalez-Sangregorio et al., 1991).

A characteristic of some open-cast lignite mine soils is the presence of lignite carbon in the parent substrate for soil development (Rumpel et al., 1998; Schafer et al., 1980; Stroo and Jencks, 1982). Often this geogenic carbon is considered to be inert (e.g. Robertson and Morgan, 1995). However, it was shown in laboratory studies that lignite can be degraded by micro-organisms (Laves et al., 1993; Fakoussa and Hofrichter, 1999; Waschkies and Hüttl, 1999). The peroxidase system responsible for lignite degradation was found to be produced by soil-inhabiting basidiomycetes (Bonnen et al., 1994) and highly stable in soil (Hofrichter et al., 1999). It was hypothesised that in the early stages of mine soil development lignite might be decomposed and thus aid the establishment of the soil

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carbon cycle (Waschkies and Hüttl, 1999). However, it may no longer be degraded when recent OM derived from plant litter is present. No studies have been carried out to examine if lignite is decomposed in mine soils where recent OM accumulated. The role of lignite in the soil carbon cycle, especially in older stages of mine soil development, is unknown.

Carbon derived from lignite and recent OM can be quantitatively estimated by ^{14}C analyses (Rumpel et al., 2000). The application of ^{14}C activity measurements to CO_2, and humic acids of lignite-containing mine soils of different ages is a tool to elucidate whether lignite takes an active part in the carbon cycle of these soils. In this study, lignite-containing mine soils were incubated for 16 months and the CO_2-emission from these soils, with and without the contribution of recent OM, was monitored. The CO_2 was trapped and analysed for its ^{14}C activity. Additionally, humic acids extracted from the soil samples used for the incubation were analysed for their content of lignite-derived compounds. The aim of the study was to examine (1) if lignite in lignite-containing mine soils is decomposed in the presence of recent OM and (2) if it is transformed during biodegradation under field conditions.

2. Material and methods

2.1. Description of the sampling sites, sampling and sample pre-treatment

Sampling was carried out at rehabilitated mine sites of Lusatia, in the eastern part of the Federal Republic of Germany. The mine spoils were rehabilitated with Scots pine (*Pinus sylvestris*, 14 years) and with red oak (*Quercus rubra,* 37 years). Prior to planting, the soils were ameliorated with alkaline ash from lignite-fired power stations and fertilised with NPK (Katzur and Haubold-Rosar, 1996). The parent substrate for soil development was sandy (64–73% sand) overburden material. After afforestation, plant-derived material (recent OM) accumulated on the soil surface and was incorporated by soil faunal activity into the mineral soil. During sampling, humic substances formed during the decomposition of plant litter could not be distinguished by macromorphological observation from lignite inherent to the parent substrate. Samples for the incubation experiment were taken from the surface mineral soil (0–15 cm) of the two lignite-containing mine soils under forests. Additionally the extremely acid (pH 3) lignite-containing parent substrate was sampled from a spoil bank where the dumped material had been deposited. The parent substrate was ameliorated with alkaline lignite-ash to increase the pH to about 6 and fertilised with NPK. All samples were homogenised, air dried and the >2 mm fraction removed by sieving. Chemical parameters of the soil samples can be found in Table 1. It appears that the samples were not really comparable because the concentration of lignite differs as well as the type of vegetation. However, it was assumed that lignite and recent organic matter behave differently in terms of decomposability so that general data on the role of lignite in the carbon cycle of soils could be obtained.

2.2. Humic acid extraction

The soil samples were extracted with 10 ml 0.25 M NaOH for 4 h at 60 °C. Afterwards the solution was acidified to pH 1 with concentrated HCl to remove the humic acids. The extraction residue was washed to pH $<$ 9.5. Then 0.25 M HCl was added (10 ml) till pH 4.5 was reached. The humic acid fraction and the extraction residue were dried at 60 °C and analysed for their ^{14}C activity.

2.3. pH, EC, and elemental composition

The pH (H_2O) and electrical conductivity (EC) values were measured with a glass electrode in the supernatant of a 1/2.5 (w vol^{-1}) soil/water suspension. Carbon and nitrogen contents were recorded with a Leco CHN 1000 analyser (precision: $\pm 0.2\%$).

2.4. Incubation and carbon mineralisation

Three replicates of each sample (ameliorated dumped material, 14 years old and 37 years old mine soil) were incubated at 20 °C for 16 months in soil microcosms as described by Siebert et al. (1998). Approximately 5–8 kg of soil were filled in a microcosm and packed until a

Table 1
Chemical parameters of incubated soils and parent substrate

	pH	EC (μS)	OC content (g kg^{-1})	N content (g kg^{-1})	C/N	Recent carbon contribution (% OC)
Ash-ameliorated fertilised parent substrate	6.38	2331	18.2±0.2	0.5±0.1	34	1.0±0.1
14 years old mine soil	6.21	560	59.8±0.6	1.4±0.1	44	11.0±0.1
37 years old mine soil	5.43	333	107.6±1.9	4.1±0.1	26	41.8±0.2

bulk density comparable to the field was achieved for each soil. Bulk density ranged between 1.2 for the 37 years old mine soil and 1.5 for the ash-ameliorated parent material. The water content was adjusted to 50% of the maximum water holding capacity (WHC) and regularly monitored and readjusted to its original value by the addition of distilled water. The soil-respired CO_2, which represents the flux of CO_2 through a soil (Cerling et al., 1991) was measured regularly by an infrared gas analyser (Rosemount, Binos 100) in a closed circulation system. For this measurement the microcosm was closed and the CO_2-concentration recorded (first reading). After some minutes the CO_2 concentration was recorded for a second time (second reading). The carbon mineralisation rate (μg CO_2–C d^{-1} g C^{-1}) was calculated after subtraction of the two values (second reading- first reading). Cumulative CO_2 production was calculated by extrapolation of the carbon mineralisation rates measured during a measuring period. The first measurements were carried out after 1 week of accommodation of the soils to laboratory conditions. To determine whether lignite was decomposed, ^{14}C measurements of the soil-respired CO_2 were carried out. Before CO_2 recovery, the circulation system including the head space was treated with synthetic air free of CO_2 to minimise contamination. Rinsing of the whole system including the soil sample is not feasible. After the synthetic air treatment a bottle with 50 ml 0.05 M NaOH, prepared with gas-free distilled water was included in the circulation system and the emitted CO_2 captured. To obtain sufficient amounts of carbon, the system was closed for 6–12 h. The CO_2 collection was carried out after 6, 12 and 16 months. All handling of the NaOH solution was carried out under N_2 to avoid contamination with CO_2 from the air.

2.5. $^{2.5.14}C$ activity measurements

Solid or liquid samples were converted to CO_2 in order to be analysed for their ^{14}C activity. The CO_2 was liberated from the 0.05 M NaOH with 40% H_3PO_4 by an automated extraction system (DICI-system) and captured in liquid nitrogen. CO_2 was obtained from solid samples by ignition at 900 °C. The CO_2 was reduced to graphite which was analysed by accelerated mass spectrometry (AMS) (Nadeau et al., 1998). The ^{14}C activity was corrected for isotopic effects after Stuiver and Polach (1977). The $\delta^{13}C$ ratio was also determined in the AMS spectrometer. The measurements were carried out in the Leibniz laboratory at the University of Kiel. The lignite content (X in%) of the sample can be estimated from the measured ^{14}C activity, $A_{meas.}$, and the ^{14}C activity of recent OM (A_{recent}):

$$X = \left[1 - \left(A_{meas.} \times A_{recent}^{-1}\right)\right] \times 100 \quad (1)$$

The ^{14}C activity of the recent OM in the sampled soils can be in the range of 120–110 pMC (percent modern carbon) (14 year old site) and 180–110 pMC (37 years old site) (Levin and Kromer, 1997). However, the lignite content of the samples was estimated with a reasonable degree of accuracy (± 10%) by using 115 pMC for recent OM (Rumpel et al., 2000).

2.6. Statistical analysis

For curve fitting a single exponential model [Eq. (1)] and a double exponential model [Eq. (2)] were used (Rovira and Vallejo, 1997):

$$A_t = A_0 \exp(-kt) \quad (2)$$

where A_t is the amount of the variable A remaining at time t, A_0 is the amount of A at zero time and k is the rate constant.

$$A_t = A_1 \exp(-k_1 t) + A_2 \exp(-k_2 t) \quad (3)$$

where the initial OM is divided into two compartments (A_1 and A_2), each one with a different decomposition rate k. A_1 is the labile compartment and A_2 is recalcitrant ($k_1 >> k_2$). Curve fitting of the mineralisation data was carried out with the computer software SIGMA PLOT 4.0 (Jandel Scientific).

3. Results and discussion

3.1. Chemical parameters

The pH values of all samples were between 5 and 6 (Table 1), showing that comparable conditions for soil micro-organisms were present in all soils. The samples were characterised by high carbon contents compared to young natural soils (Table 1). ^{14}C activity measurements of the bulk soil showed that the contribution of recent carbon ranged from 1 to 42% of the total organic carbon. In the oldest mine soil, the contribution of recent carbon was highest. This is consistent with previous results (Rumpel et al., 1999), which showed that the accumulation of recent OM and its degree of humification increase with mine soil age. The lignite carbon concentration in the three soils was found to be different which could mean that the sources of the parent materials were different. In the Lusatian mining district different concentrations of lignite in the overburden material are most probably related to the lignite-mining procedure which mixes overburden close to the lignite seams having a high lignite concentration with overburden further away from the seam having a low lignite concentration (Häge, 1996). It is unlikely that the lignite in the three soils, although being present in different concentrations, differ

in chemical composition and therefore in decomposability because the lignite seams in the Lusatian mining district occur over a large surface with a similar chemical constitution.

3.2. Carbon mineralisation

The total amounts of C released as CO_2 from the soils after 16 months of incubation was 32 ± 15 mg CO_2–C gC^{-1} for the parent substrate, 60 ± 11 mg CO_2–C gC^{-1} for the 14 years old mine soil and 31 ± 5 mg CO_2–C gC^{-1} for the 37 years old mine soil. Approximately half the carbon mineralisation occurred within the first 6 months of incubation. The cumulative CO_2 production is in the range of values found during incubation of a boreal forest soil (Ross et al., 1999). Similar amounts of carbon were released from the 37 years old mine soil and the ameliorated and fertilised parent substrate containing lignite as a single carbon source. This shows that lignite and recent OM are both available to soil microorganisms. These results are in contrast to incubations carried out by Robertson and Morgan (1995), who did not find an increase in carbon mineralisation from a soil to which brown coal was added. However, the brown coal used in their study had a high C/N ratio of 94, indicating a relatively high stage of coalification. Organic material with such a C/N ratio is usually not easily available to micro-organisms.

The carbon mineralisation rate was found to decline over the first six months of incubation and it was highest in the 14 years old soil ranging between 90 and 150 μg CO_2–C d^{-1} g C^{-1} (Fig. 1). The mineralisation rate showed a decrease during incubation as well as with increasing mine soil development from the 14 to the 37 years old soil. A decrease of microbiological activity with increasing age of mine soils was also observed by other authors (Insam and Domsch, 1988; Stroo and Jencks, 1982) and explained by the accumulation of compounds which are stabilised against decomposition. During soil development the degree of humification of recent OM was found to increase in lignite-containing mine soils (Rumpel et al., 1999), showing that stabilisation of recent organic matter against decomposition occurred from the 14 to 37 years old soil. The decomposition pattern followed a single exponential equation in the parent substrate with lignite as a single carbon source, the rate constant being 0.01 d^{-1} (Fig. 1). In the 14 and 37 years old mine soil, the decomposition pattern was best explained by a double exponential model assuming two OM compartments with different decomposition rates. The labile compartment had a rate constant of 0.24–0.21 d^{-1}. Those values are in the range of

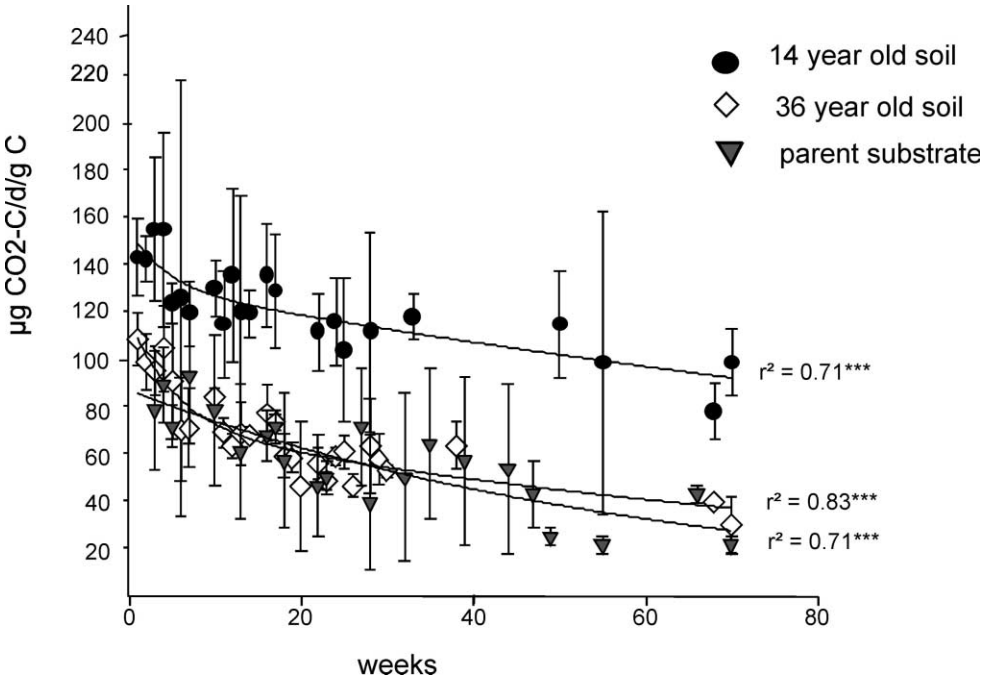

Fig. 1. Carbon mineralisation rate (μg CO_2–C d^{-1} g^{-1} C) during 16 months of incubation. Curve fitting yielded the following equations:

Parent substrate	$y = 1.50 \times \exp(-0.01t)$
14 years old soil	$y = 1.43 \times \exp(-0.24t) + 7.8 \times \exp(-0.005t)$
37 years old soil	$y = 4.30 \times \exp(-0.21t) + 7.8 \times \exp(-0.012t)$.

rate constants found by Ajwa and Tabatabai (1994) who examined the decomposition of recent OM types such as plant litter, compost, and sewage sludge in soils. The more refractory compartment which decomposed more slowly had a rate constant of 0.005 and 0.012 d^{-1} in the 14 and 37 years old mine soil. This compartment could be related to lignite, in agreement with results of other authors who found that lignite is not easily decomposed by microorganisms (e.g. Robertson and Morgan, 1995). Based on a comparison of the three soils it could be suggested that the rate constant of the refractory compartment increases with the time of exposure in the soil. In contrast the mineralisation rate of lignite was found to decrease with time (see below). To explain this discrepancy, we suggest that the refractory compartment is not exclusively related to lignite. Our results show that lignite in mine soils is subject to considerable mineralisation, part of it following the high rate constant and part of it being refractory. Waschkies and Hüttl (1999) hypothesised that the decomposition of lignite may be less important in older mine soils when the accumulation of OM derived from plant material occurred. To examine the degradability of lignite in mine soils of different ages, ^{14}C activity measurements of the soil-respired CO_2 from the 14 and 37 years old mine soil were carried out.

3.3. ^{14}C activity measurements of the soil-respired CO_2

The lignite contribution ranged from 24% of CO_2 evolved from the 37 years old mine soil up to 63% of the CO_2 evolved from the 14 years old mine soil. In the lignite-containing parent substrate a maximum of 61% of a lignite contribution to the CO_2–C was observed after 6 months (Table 2). The relatively low contribution of lignite carbon to the CO_2 emitted from a soil containing lignite as a single carbon source can be explained by the sampling procedure. Although all handling of the NaOH solution was carried out under N_2 to avoid contamination with CO_2 from air, recent carbon was trapped. This is due to the fact that the soil sampled was not CO_2-free, but included CO_2 from soil air because during sampling only CO_2 in the head-space was eliminated. The CO_2 in soil air is not entirely derived from microbial respiration. Microbial respired CO_2 mixes with atmospheric CO_2 (Galimov, 1966) which results in a specific isotopic composition depending on how much atmospheric CO_2 mixes with microbial respired CO_2 (Amundson et al., 1998). To estimate quantitatively lignite mineralisation the lignite contribution to the CO_2 emission was normalised to the lignite content in the soil (lignite mineralisation rate: μg lignite CO_2–C d^{-1} g lignite C^{-1}).

The lignite mineralisation rate was highest in the 14 years old soil where 65–74 μg lignite CO_2–C d^{-1} g lignite C^{-1} were recorded (Table 2). The average decay rate of lignite in the 14 and 37 years old sites was calculated as 0.025 and 0.007 g lignite C kg^{-1} C $year^{-1}$ respectively. These mineralisation rates are comparable to those recorded for a whole range of natural soil OM (Jörgensen, 1995). The average decay rate of lignite shows that lignite is decomposed to a greater extent on the younger site. The decrease in lignite mineralisation with soil age may be due to a loss of easily decomposable lignite structures due to decomposition and relative accumulation of more stable compounds, as noticed for recent OM (Stroo and Jencks, 1982). However, many processes can influence the average decomposition rate including protection of lignite by recent OM, the effect of vegetation (pine vs oak) or of changes in physical properties and nutritional status of the soil on microbial populations via phenolic compounds or mycorrhizae.

The decomposition of lignite was observed in laboratory experiments by many authors (e.g. Willmann and Fakoussa, 1997; Waschkies and Hüttl, 1999) and the transformation pathways are known. The microbial degradation of lignite is carried out by unspecific extracellular attacks similar to the degradation of lignin including oxidative enzymes, hydrolytic enzymes,

Table 2
Lignite contribution to the total CO_2 emission and lignite mineralisation during incubation

Soil sample	Lignite contribution to the total CO_2 emission (% lignite-CO_2–C total CO_2—C^{-1})		
	6 months	12 months	16 months
Parent substrate	60.7±0.1[a]	50.5±0.2	26.7±0.3
14 years old mine soil	59.9±0.2	62.8±0.2	60.2±0.2
37 years old mine soil	24.4±0.2	28.1±0.2	26.7±0.2
	Lignite mineralisation (μg lignite CO_2–C d^{-1} g lignite-C^{-1})		
Parent substrate	24.5±7.2	13.6±7.6	9.9±4.2
14 years old mine soil	74.4±27.8	68.3±44.2	65.1±9.5
37 years old mine soil	22.0±6.9	26.2±6.1	11.8±4.5

[a] The standard deviation relates to the ^{14}C activity measurements.

Table 3
Carbon recovery, ^{14}C activity and percentage of carbon extractable as humic acids from recent plant litter and lignite

	Carbon recovery in humic acid (%)	^{14}C activity of humic acid (pMC*)	C in humic acids extractable from recent carbon (%)	C in humic acids extractable from lignite[a] (%)
37 years old soil	58.4	37.00±0.16	54	64
14 years old soil	28.6	6.96±0.08	15	29
parent substrate	31.8	0.54±0.06	5	30

* Percentage modern carbon.
[a] Caculated based on the lignite content in the soil.

alkaline metabolites and natural chelators (Fakoussa and Hofrichter, 1999). However, the high and versatile potential activities of micro-organisms to degrade coals seems to be in contrast to observations under natural conditions (Fritsche et al., 1998). To test whether lignite is transformed under field conditions humic acid extraction of the samples used for the incubation was carried out.

3.4. Humic acid extraction

The highest amounts of humic acids could be extracted from the 37 years old mine soil (Table 3) amounting to 58% compared to about 30% in the lignite-containing parent substrate and the 14 years old mine soil. The increase in humic acid yields with increasing soil age may be, in part, related to the different tree species present on the sites. However, it could also be explained by the higher degree of humification recorded for recent OM in the oldest mine soil. It is striking that the ^{14}C activity of the humic acids extracted from the oldest soil was only 37 pmC. This means that in older soils a higher proportion of humic acids was derived from lignite. The proportion of humic acids derived from lignite increased from 30 to 64% with increasing soil age (Table 3). This means that both lignite and plant litter are altered by humification processes in mine soils. The oxidation of lignite supposedly leads to the formation of carboxylic groups which enhances the extraction of these compounds by NaOH (Kögel-Knabner, 1993; Haider, 1999). These results are in agreement with a study by Chang and Berner (1998), who found a marked increase of carbonyl groups after laboratory oxidation of subituminous coal. The humic acid fraction consists of a mixture of many kinds of material, e.g. microorganisms, plant litter, humified compounds and even pyrogenic material where present in soil (Oades, 1988; Skjemstad et al., 1996). Despite these drawbacks, humic acid extraction may be useful to observe changes during biodegradation. The proportion of carbon that can be extracted as humic acids from the mine soils with increasing soil age is related to an increasing transformation of the OM (Table 3). Therefore, the analysis of humic acids, although operationally defined, in combination with ^{14}C activity measurements provides evidence for lignite transformation in mine soil under field conditions.

4. Conclusion

Incubation for 16 months of lignite-containing mine soils showed that lignite is decomposed in the lignite-containing parent substrate as well as in the presence of recent OM in older mine soils. ^{14}C activity measurements of soil-respired CO_2 indicated the highest lignite decomposition in the 14 years old mine soil. Lignite decomposition decreased with increasing age of the soil. ^{14}C activity measurements of the humic acid fraction of the soil samples used for incubation showed that lignite was transformed during biodegradation. This transformation includes oxidative decomposition (formation of CO_2 and humic acids) which is in line with the oxidative processes of lignite biodegradation reported after in vitro studies.

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