Pedogenic carbonates in loess

formation rates, formation conditions and source apportionment assessed by isotopes and molecular proxies

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In Memoriam

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Abbreviations

^{13}C	stable carbon atom with atomic mass 13
^{14}C	radioactive carbon atom with atomic mass 14
ACL	average chain length
AMS	accelerator mass spectrometer
ANOVA	analysis of variance
a.s.l.	above sea level
B.P.	before present (1950)
Bq	Becquerel [decays second^{-1}]
BSTFA	*N,O*-bis(trimethylsilyl)trifluoroacetamide
C	carbon
C_3 plants	plants following the C_3-photosynthesis metabolism (e.g. ryegrass, wheat)
C_4 plants	plants following the Hatch-Slack photosynthesis metabolism (e.g. maize)
C_{carb}	carbonatic C
C_{org}	organic C
C_t	total carbon
CPI	carbon preference index
DCM	dichloromethane (H_2CCl_2)
DIC	dissolved inorganic carbon (CO_2(aq), HCO_3^- and CO_3^{2-} from dissolved CO_2 of root and rhizomicrobial respiration)
DOC	dissolved organic carbon (root exudates and their microbial metabolites)
FA	fatty acid
GC-FID	gas chromatograph with flame ionization detector
GS	growing season
IRMS	isotope ratio mass spectrometer
L	reference loess
LCFA	long chain fatty acid
LCA	long chain alkane
LOM	loess organic matter
LSC	liquid scintillation counting
M	molar [mol l^{-1}]
MUFA	mono-unsaturated fatty acids
NA	natural abundance of an isotope
n-C_x	unbranched aliphatic hydrocarbon with x carbon atoms
OM	organic matter
PDB	PeeDee Belemnite
PUFA	poly-unsaturated fatty acids
RL	rhizosphere loess
RR	recrystallization rate [day^{-1}]
SEM	standard error of the mean
SIC	soil inorganic carbon
SOM	soil organic matter
SPE	solid phase extraction
SQP(E)	external Standard Quench Parameter
SQP(I)	internal Standard Quench Parameter
WHC	water holding capacity [%]

Summary

Interest in secondary (pedogenic) carbonates as an archive for paleoclimatic reconstructions in soils and sediments of arid and semiarid climates has increased during recent decades. Their carbon (C) isotope composition represents the conditions prevailing during their formation because they are formed by precipitation of Ca^{2+} from soil solution with dissolved CO_2 from soil air originating from root and rhizomicrobial respiration. Thus, pedogenic carbonates are an important tool for estimation of age of pedogenesis and for reconstruction of the local paleovegetation. Potential reequilibration of pedogenic carbonates with younger soil CO_2 can entail loss of chronological and paleoenvironmental information. Although methodological resolution of these studies depends on the time scale of pedogenic carbonate formation and recrystallization, its rates and periods remain unknown.

The first objective therefore was the first-time assessment of the time frame of pedogenic $CaCO_3$ formation and recrystallization under controlled conditions, and its dependance on environmental factors and position in the soil profile. The other aim was to reveal the potential of rhizoliths, a special form of pedogenic carbonates (calcified roots), from a SW German loess-paleosol sequence for paleoenvironmental studies by elucidation of source vegetation and chronological context.

In loess as a common soil parent material, initial $CaCO_3$ recrystallization rates were successfully determined with the ^{14}C isotopic exchange approach by exposing loess to artificially labeled $^{14}CO_2$ and subsequent quantification of ^{14}C incorporated in secondary (recrystallized) $CaCO_3$. Within the range of natural soil CO_2 concentrations, recrystallization rates depended strongly on CO_2 concentration, with highest values at highest CO_2 concentration. This suggested that recrystallization rates in planted soil with permanently high CO_2 concentration should exceed those in unplanted soil, and that fastest recrystallization should occur in rhizosphere. In further studies, loess was exposed to $^{14}CO_2$ respired by roots and rhizomicrobial organisms of plants labeled in $^{14}CO_2$ atmosphere to estimate the effects of further factors (root vicinity, temperature, accumulation depth) on the recrystallization rate. Rates from planted loess were two orders of magnitude higher than those from unplanted loess, mostly in the range of 10^{-5} day^{-1}, i.e. 10^{-3} % of loess $CaCO_3$ were recrystallized per day. Significantly higher $CaCO_3$ recrystallization rates in rhizosphere than in loess distant from roots were attributed to three factors: high CO_2 concentration from root and rhizomicrobial respiration, low pH caused by release of CO_2 and root exudates, and high Ca^{2+} and HCO_3^- concentration caused by water uptake by roots. Considerable influence of the latter was demonstrated by low $CaCO_3$ recrystallization rates at low temperatures and *vice versa*, reflecting the increasing transpirational pull with increasing temperatures. Assuming repeated recrystallization of both primary and secondary $CaCO_3$, extrapolation of initial $CaCO_3$ recrystallization rates showed that at least 10^2 – 10^3 years are necessary for complete recrystallization of $CaCO_3$ in 'root-free' loess by formation of secondary $CaCO_3$, depending on length of the growing season. Increasing temperature promoted $CaCO_3$ recrystallization rates, but the contrast between low and high temperatures was compensated for

recrystallization periods because of the negative effect of increasing temperature on length of the growing season. In contrast, pedogenic carbonates can form much faster close to roots (10^1 – 10^2 years) because of mass flow to the roots leading to formation of concretions (rhizoliths). As a consequence of this wide temporal spectrum of pedogenic carbonate formation, variable methodological resolution has to be considered in paleoenvironmental studies based on stable isotope composition of pedogenic carbonates, depending on climatic factors and formation of carbonate concretions.

Rhizoliths, formed by encrustation of roots with secondary $CaCO_3$, yield high potential for paleoenvironmental studies. At the late Pleistocene loess-paleosol sequence of Nussloch, SW Germany, rhizolith $CaCO_3$ was completely secondary and not contaminated by postsegregational alteration. Radiocarbon dating of one rhizolith sample reinforced the assumption of potential postsedimentary formation of rhizoliths. In the investigated profile, stable C isotope composition indicated C_3 source vegetation for organic matter (OM) of both loess and rhizoliths, but lipid molecular proxies revealed grass above- and belowground biomass as origin of loess OM, and shrub or tree roots as source of rhizoliths. Moreover, OM in loess adjacent to rhizoliths was considerably contaminated by rhizomicrobial and root remains at least up to a distance of 5 cm. Alteration of loess OM and its isotope composition by postsedimentary penetration of deep-rooting plants might entail uncertainties for paleoenvironmental studies based on loess OM.

In summary, the important role of vegetation on pedogenic $CaCO_3$ formation and recrystallization was shown under controlled and field conditions. Roots and associated microorganisms have direct influence on these processes, while further factors of pedogenesis like climate exert an indirect effect, but on the long term probably are of greater importance than effects on the rhizosphere scale.

Zusammenfassung

In den letzten Jahrzehnten hat das Interesse an pedogenen (sekundären) Karbonaten als Archiv für Paläoklimarekonstruktionen in Böden und Sedimenten arider und semiarider Regionen zugenommen. Die Kohlenstoffzusammensetzung pedogener Karbonate spiegelt die Bedingungen zur Zeit ihrer Bildung wider, da sie durch Ausfällung von Ca^{2+} aus der Bodenlösung mit gelöstem CO_2 der Bodenluft entstehen, wobei letzteres hauptsächlich aus Wurzel- und rhizomikrobieller Atmung stammt. Deshalb sind pedogene Karbonate ein wichtiger Schlüssel zur Bestimmung des Alters der Bodenbildung und zur Rekonstruktion der lokalen Paläovegetation. Potentielle spätere Reäquilibrierung pedogener Karbonate mit jüngerem Boden-CO_2 kann zu einem Verlust der chronologischen und paläoökologischen Informationen führen. Obwohl die methodische Auflösung dieser Studien vom Zeitrahmen der Bildung und Rekristallisation pedogener Karbonate abhängt, sind deren Raten und Zeiträume bisher unbekannt.

Das erste Ziel war es daher, erstmalig den Zeitrahmen der Bildung und Rekristallisation pedogener Karbonate so wie dessen Abhängigkeit von Umweltfaktoren und der Position im Bodenprofil unter Laborbedingungen zu ermitteln. Weiteres Ziel war, die potentielle Eignung von Rhizolithen, einer besonderen Form von pedogenen Karbonaten (verkalkte Wurzeln) aus einer südwestdeutschen Löss-Paläosol-Sequenz für Paläoumweltstudien aufzuzeigen. Hierfür wurde die Ursprungsvegetation der Rhizolithe und deren chronologischer Kontext untersucht.

In Löss, einem verbreiteten Ausgangsmaterial der Bodenbildung, wurde der Ansatz des ^{14}C-Isotopenaustausch erfolgreich angewandt, um initiale $CaCO_3$-Rekristallisationsraten zu bestimmen. Hierzu wurde der Löss künstlich markiertem $^{14}CO_2$ ausgesetzt, und der durch Rekristallisation in das $CaCO_3$ eingebaute ^{14}C-Tracer anschließend quantifiziert. Im Bereich der natürlich in Böden auftretenden CO_2-Konzentrationen zeigten die Rekristallisationsraten eine starke Abhängigkeit von der CO_2-Konzentration, wobei unter der höchsten CO_2-Konzentration die höchste Rate auftrat. Dies ließ vermuten, dass Rekristallisationsraten in von Pflanzen besiedeltem Boden durch permanent hohe CO_2-Konzentration höher sind als jene in unbepflanztem Boden, und dass die Rekristallisation in der Rhizosphäre am schnellsten stattfindet. In weiteren Studien wurde Löss dem von Wurzeln und Rhizomikroorganismen veratmeten $^{14}CO_2$ ausgesetzt, nachdem die Pflanzen in $^{14}CO_2$-Atmosphäre markiert worden waren. Hierdurch konnte der Einfluss weiterer Faktoren wie Wurzelnähe, Temperatur und Akkumulationstiefe auf die Rekristallisationsrate bestimmt werden. Die Raten in bepflanztem Löss lagen zwei Größenordnungen über jenen in unbepflanztem Löss, überwiegend im Bereich 10^{-5} Tag^{-1}, d.h. je Tag wurden 10^{-3} % des Löss-$CaCO_3$ rekristallisiert. Signifikant höhere $CaCO_3$-Rekristallisationsraten in der Rhizosphäre als in wurzelfernem Löss wurden auf drei Faktoren zurückgeführt: hohe CO_2-Konzentration durch Wurzel- und mikrobielle Atmung, niedriger pH-Wert aufgrund der Abgabe von CO_2 und Wurzelexudaten und hohe Ca^{2+}- und HCO_3^--Konzentration, hervorgerufen durch Wasseraufnahme der Wurzeln. Der bedeutende Einfluss des letzten Faktors wurde dadurch bestätigt, dass $CaCO_3$-Rekristallisationsraten unter niedrigen Temperaturen am geringsten waren und umgekehrt.

Dies spiegelt den mit steigender Temperatur zunehmenden Transpirationssog wider. Unter der Annahme mehrmaliger Rekristallisation des primären und sekundären $CaCO_3$ zeigt die Extrapolation der initialen $CaCO_3$-Rekristallisationsraten, dass im wurzelfernen Löss mindestens 10^2 – 10^3 Jahre für die vollständige Rekristallisation des $CaCO_3$ durch Bildung pedogener Karbonate notwendig sind. Die Dauer wird durch die Länge der Vegetationszeit bedingt. Zunehmende Temperatur erhöhte zwar die $CaCO_3$-Rekristallisationsraten, aber der Kontrast zwischen geringen und hohen Temperaturen wurde für die Rekristallisationsperioden durch den negativen Effekt steigender Temperaturen auf die Länge der Vegetationszeit abgeschwächt. Im Gegensatz dazu können pedogene Karbonate in Wurzelnähe viel schneller gebildet werden (10^1 – 10^2 Jahre), wenn zur Wurzel gerichteter Massenfluss zur Bildung von Konkretionen (Rhizolithen) führt. Infolge dieses weiten zeitlichen Spektrums der Bildung pedogener Karbonate muss in Paläoumweltstudien, die auf der stabilen Isotopenzusammensetzung pedogener Karbonate basieren, eine unterschiedliche methodische Auflösung berücksichtigt werden. Diese hängt von klimatischen Faktoren und der Bildung von Karbonatkonkretionen ab.

Rhizolithe, die durch Umkrustung von Wurzeln mit sekundärem $CaCO_3$ entstehen, weisen ein hohes Potential für Paläoumweltrekonstruktionen auf. In der spätpleistozänen Löss-Paläoboden-Sequenz von Nussloch (SW-Deutschland) war das $CaCO_3$ der Rhizolithe vollständig sekundär und nicht durch nachträgliche Prozesse kontaminiert. Die Radiokohlenstoffdatierung eines Rhizoliths bekräftigte die Vermutung einer möglichen postsedimentären Bildung der Rhizolithe. Im untersuchten Profil deutete sowohl die stabile Kohlenstoffzusammensetzung der organischen Substanz (OM) im Löss als auch die der Rhizolithe auf C_3-Pflanzen als Ursprungsvegetation hin. Molekulare Lipidproxies zeigten jedoch, dass die Lössorganik von ober- und unterirdischer Grasbiomasse stammt, während die Rhizolithe von Strauch- oder Baumwurzeln gebildet wurden. Darüber hinaus war die OM im den Rhizolithen benachbarten Löss bis in eine Distanz von mindestens 5 cm deutlich durch rhizomikrobielle und Wurzelüberreste kontaminiert. Die Überprägung der Lössorganik und ihrer isotopischen Zusammensetzung durch postsedimentär eindringende tiefwurzelnde Pflanzen könnte die Ergebnisse von auf Lössorganik basierenden Paläoumweltstudien verfälschen.

Zusammenfassend wurde die Bedeutung der Vegetation für die Bildung und Rekristallisation pedogener Karbonate unter Labor- und Feldbedingungen gezeigt. Wurzeln und zugehörige Mikroorganismen haben direkten Einfluss auf diese Prozesse, während weitere Faktoren der Bodenbildung, z.B. Klima, einen indirekten Einfluss ausüben. Über längere Zeiträume sind diese Faktoren aber wahrscheinlich wichtiger als Effekte auf Rhizosphärenskala.

I. Extended Summary

1 Introduction

1.1 Object of research

Pedogenic carbonates are a typical feature of soils of arid and semiarid regions (Birkeland 1999, Eswaran et al. 2000), with calcium carbonate ($CaCO_3$) as the main carbonate-containing phase (Doner and Lynn 1989). Apart from soft diffuse masses (e.g. Kovda et al. 2006), pedogenic carbonates occur in a variety of discrete forms, e.g. pseudomycelia (Alonso et al. 2004, Łącka et al. 2009), coatings on the lower side of clasts (Courty et al. 1994, Pustovoytov 2003), soft or hard nodules (Khokhlova et al. 2008), calcified roots (Klappa 1980, Pustovoytov and Terhorst 2004), calcretes (Freytet et al. 1997, Grevenitz 2006). In moderate to mature soil profiles, these are typically accumulated within soil horizons (Gile et al. 1966, Birkeland 1999). For differentiation from the primary (lithogenic) carbonate of soil parent material, pedogenic carbonates are frequently called secondary carbonate. Herein, the term 'pedogenic carbonate' refers to soil carbonates that form in the vadose zone and does not comprise those carbonates that form at the top of the water table (e.g. groundwater calcrete) or in transitionary zones (e.g. palustrine carbonates; Alonso-Zarza 2003).

Pedogenic carbonates form by precipitation of Ca^{2+} present in the soil solution with soil air CO_2 dissolved as HCO_3^- (Eq. 1, arrow to the left side). In calcareous parent material, this process involves dissolution of primary $CaCO_3$ (Eq. 1, arrow to the right side).

$$CaCO_3 + CO_2 + H_2O \rightleftharpoons Ca^{2+} + 2\ HCO_3^- \quad (1)$$

The doctrine that the resulting pedogenic carbonate comprises one half carbon (C) from primary carbonate and the other half C from soil air CO_2 has been disproved by Cerling (1984). According to the model of Cerling (1984) based on stable C isotope composition ($\delta^{13}C$), C in pedogenic carbonates originates completely from soil air CO_2. With the latter being released predominantly by root respiration and microorganisms decomposing rhizodeposits and soil organic matter (SOM; Amundson et al. 1998), the relation between stable C isotope composition of pedogenic carbonate ($\delta^{13}C_{pedogenic}$) and that of vegetation present during its formation ($\delta^{13}C_{vegetation}$) can be described as follows:

$$\delta^{13}C_{pedogenic} = \delta^{13}C_{vegetation} + \nu + \varepsilon \quad (2)$$

where ν = 4.4 ‰ is the isotopic fractionation by molecular diffusion of CO_2 (O'Leary 1981, Cerling and Quade 1993, Nordt et al. 1998), and ε is temperature dependant isotopic fractionation by carbonate equilibrium reactions (Romanek et al. 1992). Assuming an average value for ε of 10.5 ‰ (Koch 1998), this results in secondary carbonate $\delta^{13}C$ values which are ~ 14.9 ‰ higher than those of the corresponding plant biomass.

1.2 Pedogenic carbonates in paleoenvironmental studies

In arid and semiarid environments, pedogenic carbonates have been used as a tool to assess conditions that prevailed during soil formation based on carbonate morphology

(e.g. Kemp 1995, Becze-Deák et al. 1997) as well as depth and intensity of carbonate accumulation (e.g. Gile et al. 1966, Machette 1985).

However, it was not until recovery of the C isotopic relation between pedogenic carbonates and soil CO_2 (Cerling 1984) that quantitative paleoenvironmental studies based on $\delta^{13}C$ of pedogenic carbonates became possible. Main aim of these paleoenvironmental reconstructions is determination of paleovegetation with respect to its photosynthetic pathway. Mechanisms of isotopic fractionation by C_3 (trees, shrubs and grasses of temperate climates) and C_4 plants (mostly grasses of arid climates) is well known (Boutton 1996, Cerling and Harris 1999), with average $\delta^{13}C$ values of –27 ‰ (range –19 to –36 ‰) for C_3 and –13 ‰ (range –9 to –16 ‰) for C_4 vegetation. This isotopic signal is incorporated in pedogenic carbonates via root respiration and microbial decomposition of SOM. Subsequently, average $\delta^{13}C$ values of pedogenic carbonates derived from pure C_3 or C_4 vegetation approximate –12 ‰ or +2 ‰ (Fig. I).

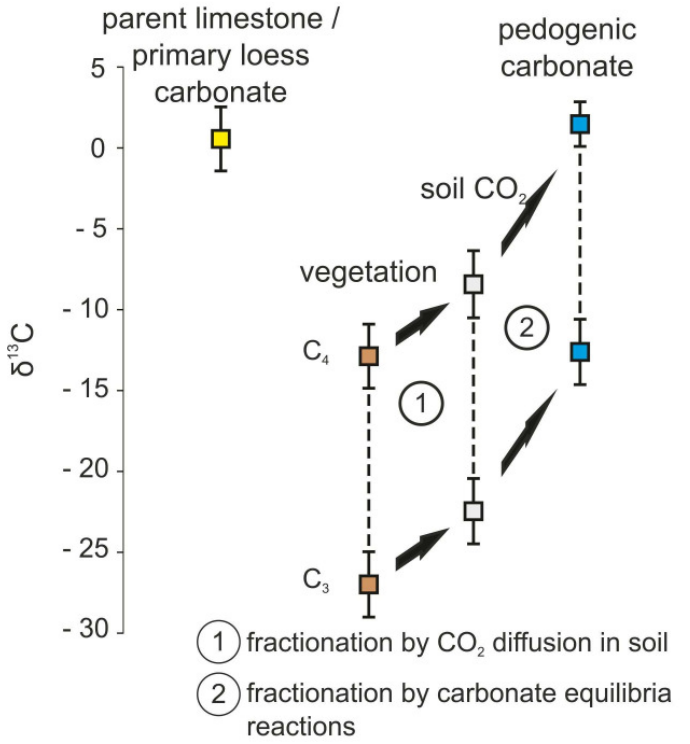

Figure I: Relationships between stable carbon isotope composition of vegetation, soil CO_2 and pedogenic carbonate. In the majority of soils, $\delta^{13}C$ of pedogenic carbonate is a function of $\delta^{13}C$ of soil CO_2 released by root respiration and microorganisms decomposing SOM (redrawn from Nordt et al. 1996).

In sediment-paleosol sequences, upper soil horizons, in contrast to more resistant Bk horizons, are often truncated (Cojan et al. 2000), or C_{org} content is too low for $\delta^{13}C$ measurement. Therefore, pedogenic carbonates are used to assess relative portions of C_3 vs. C_4 vegetation which is influenced by climate (temperature, aridity) and atmospheric CO_2 concentration (Ehleringer et al. 1997). In numerous studies, C_4 expansion events and shifts from C_4 towards C_3 vegetation could be identified in loess-paleosol sequences (Quade and

Cerling 1995, Ding and Yang 2000) as well as other sedimentary archives (Liu et al. 1996, Monger et al. 1998, Buck and Monger 1999, Fox and Koch 2003). Based on soil $CaCO_3$ isotope composition, a more detailed characterization of paleovegetation communities concerning plant habits was tried (Cerling 1992, Quade and Cerling 1995, Liu et al. 1996), partly in combination with pollen data (e.g. Monger et al. 1998). However, at sites with continuous C_3 vegetation, such hypotheses are difficult to draw solely from isotope composition, as the group of C_3 plants comprises grasses as well as shrubs and trees. Additionally, uncertainties remain with respect to carbonate precipitation on the rhizosphere scale (Monger et al. 2009). Many paleoenvironmental studies combine stable carbon and oxygen isotope composition ($\delta^{18}O$), because $\delta^{18}O$ values of pedogenic carbonates are related to the isotopic signal of meteoric water and therefore provide a record of paleotemperatures, precipitation amount, paleopatterns of atmospheric circulation or a combination of these parameters (Dworkin et al. 2005, Monger et al. 1998). As an example, Łącka et al. (2009) analyzed pedogenic carbonates in loess-paleosol sequences of Eastern Europe for $\delta^{13}C$ and $\delta^{18}O$ to assess paleoclimatic conditions. In recent times, the 'clumped isotope approach' was applied to pedogenic carbonates to use them as a paleothermometer (Ghosh et al. 2006). Further, based on the diffusion-reaction model developed by Cerling (1984), $\delta^{13}C$ of pedogenic carbonates is used to estimate former atmospheric CO_2 concentration (Cerling 1992, Mora et al. 1996). In summary, $\delta^{13}C$ and $\delta^{18}O$ of pedogenic carbonates allows to draw conclusions about paleovegetation and former climatic conditions including paleotemperature, paleoprecipitation, moisture conditions and atmospheric pCO_2 in arid and semiarid environments. Additionally, pedogenic carbonates are used to determine age of pedogenesis and sediments by radiocarbon dating (Chen and Polach 1986, Amundson et al. 1994, Wang et al. 1994), U/Th dating (Sharp et al. 2003) and growth rates of coatings (Vincent et al. 1994, Pustovoytov 2003).

Basic prerequisite for the above mentioned paleoenvironmental and geochronological studies interpreting isotope composition of pedogenic carbonates is long-term preservation of the isotopic signal which was incorporated in pedogenic carbonates during their formation. This is mostly true for buried soils and / or arid environments (Cerling 1991, Amundson et al. 1994, Quade et al. 1994, Lee and Hisada 1999, Royer et al. 2001). However, possible recrystallization and isotopic reequilibration of existing pedogenic carbonates with younger soil CO_2 causes an overprint of the original isotope composition (Bowler and Polach 1971, Cerling 1991, Amundson et al. 1994, Budd et al. 2002). Moreover, the temporal resolution of carbon isotopic studies on pedogenic carbonates is limited not by instrumental precision (IRMS for stable isotopic analyses, AMS for radiocarbon dating), but by the timeframe of pedogenic carbonate formation (Royer et al. 2001). Therefore, emphasis should be placed on understanding the processes of secondary carbonate formation and postsegregational alteration, as well as estimating the yet unknown rates of these processes.

1.3 Previous approaches to estimate rates of pedogenic carbonate formation and accumulation

The timeframe of accumulation of considerable amounts of soil carbonate was estimated in several studies (e.g. Landi et al. 2003), a substantial part of which was performed in Southwestern USA (Machette 1985, Schlesinger 1985, Marion 1989). These rates were calculated indirectly, dividing the carbonate content by the age of the soil, with the latter determined by independent methods (e.g. based on archeological data or by numerical dating). However, these data show several shortcomings: First, most of these studies were performed on soils developed on non-calcareous parent materials, with rainfall and dust as main sources for Ca^{2+} influx (Birkeland 1999). Second, these studies do not distinguish secondary $CaCO_3$ formation rates from accumulation rates. Accumulation rates are not simply a function of the (dissolution and) precipitation rate(s) of calcite, which sum up to the formation rate, but depend on water availability in the soil profile which in turn results from rainfall and soil permeability (Arkley 1963). Third, calculated accumulation rates are often based on the final stage of $CaCO_3$ accumulation, i.e. continuous layers composed of secondary carbonate (K horizons, calcretes; Gile et al. 1966, Birkeland 1999), and do not include non-continuous pedogenic carbonates (coatings, nodules, calcified roots). Accumulation rates were obtained mainly from mature soil profiles (> 75 kys) because presumably meaningful rates can be obtained only over long time periods (Machette 1985). As a consequence, accumulation rates determined in the field are average rates over large time periods during which climatic conditions, thus $CaCO_3$ formation rates, varied significantly (Gile et al. 1981, McFadden and Tinsley 1985). Hence, they are not suitable to estimate initial rates of pedogenic carbonate formation. In summary, rates of $CaCO_3$ formation and recrystallization in soils are unknown so far (Kuzyakov et al. 2006).

One possibility to estimate carbonate formation and recrystallization rates is analyses of carbon isotope composition ($\delta^{13}C$, Nordt et al. 1998; ^{14}C, Pendall et al. 1994) of total (primary and secondary) soil carbonate within a chronosequence of known age. However, the main obstacle using ^{13}C natural abundance (NA) is too low sensitivity for differentiation of small amounts of C involved in isotopic exchange. Radiocarbon dating provides higher methodological resolution but fails to yield valuable results because of low chronological resolution of analyzed chronosequences and lacking knowledge about initial ^{14}C content of primary carbonate.

A second approach, introduced by Kuzyakov et al. (2006), makes use of the isotopic exchange during $CaCO_3$ recrystallization (ch. 1.1) under controlled conditions. Recrystallization rates of loess $CaCO_3$ were estimated based on recovery of ^{14}C in loess carbonate, incorporated during recrystallization with rhizosphere CO_2 respired by plants which were labeled in artificial $^{14}CO_2$ atmosphere. To the best of my knowledge, the isotopic exchange approach is the only possibility to assess initial $CaCO_3$ formation rates. The method was applied in the here presented work and is explained below (ch. 2).

1.4 Definition of the term 'recrystallization'

In soils formed on limestone, mechanical admixture of lithogenic carbonate can cause a mixed isotopic signal of old and younger C in pedogenic $CaCO_3$ (Amundson et al. 1989, Monger et al. 1998). When regarding sedimentary environments, in contrast, most authors agree that precipitation of pedogenic carbonates does not involve significant amounts of CO_3^{2-} from primary material (e.g. Cerling 1984, Quade et al. 1989; ch. 1.1). However, the prerequisite for this process is the presence of Ca^{2+} in the soil solution, derived either from external (dust, rainfall) or internal sources (weathering of Ca bearing minerals in parent material; Birkeland 1999). In case of calcareous soil parent material like e.g. loess, Ca^{2+} is provided from dissolution of primary loess $CaCO_3$, meaning that loess $CaCO_3$ is dissolved and, after C isotopic exchange with soil air CO_2 and subsequent drying of soil, reprecipitated as pedogenic $CaCO_3$ (Eq. 3). This means that rates of pedogenic carbonate formation in loess are limited by $CaCO_3$ weathering rates rather than by Ca^{2+} influx rates.

$$CaC^{L}O_3 + C^{R}O_2 + H_2O \rightleftharpoons Ca(HC^{L}O_3)^{+} + HC^{R}O_3^{-} \rightleftharpoons$$
$$CaC^{L}O_3 + C^{R}O_2 + H_2O \rightleftharpoons Ca(HC^{R}O_3)^{+} + HC^{L}O_3^{-} \rightleftharpoons Ca^{R}CO_3 \downarrow + C^{L}O_2 + H_2O \qquad (3)$$

As the presented work is concerned with pedogenic carbonate formation in loess and all modelled data refer to the conditions of initial pedogenesis in loess, the term recrystallization (as well as recrystallization rate, recrystallization period) is used in the following to express the dissolution of loess $CaCO_3$ and repricipitation of secondary $CaCO_3$, which includes C isotopic exchange of primary loess $CaCO_3$ with CO_2 released by root and rhizomicrobial respiration.

1.5 Objectives

In general, the aim of this work was to contribute to a better understanding of the processes of pedogenic carbonate formation and postsegregational alteration, as well as the influence of involved soil formation factors like climate and vegetation. Especially assessment of process rates under natural conditions is not possible so far, an issue which is of great importance for several paleoenvironmental and chronological studies based on isotope composition of pedogenic carbonates.

Therefore, the methodological objective of my dissertation was to

(1) evaluate the potential of ^{14}C and ^{13}C tracers for the isotopic exchange approach to estimate initial recrystallization rates under controlled conditions.

Thereafter, the optimal C tracer should be applied in labeling experiments to

(2) determine recrystallization rates of pedogenic carbonates under controlled conditions – based on isotopic exchange between primary $CaCO_3$ and rhizosphere CO_2 – during the initial phase of pedogenesis,

(3) assess the influence of several environmental factors –

- soil CO_2 concentration

- plant species
- distance to roots
- temperature and
- $CaCO_3$ migration – on the initial $CaCO_3$ recrystallization rate,

(4) extrapolate the estimated $CaCO_3$ recrystallization rates on longer time periods for estimation of the time periods necessary for complete recrystallization of total $CaCO_3$ by formation of secondary carbonate. Modelled time periods were then discussed in the context of literature data concerning the timeframe of $CaCO_3$ formation and postsegregational recrystallization in soil.

Another key aspect of this work was precipitation of secondary $CaCO_3$ in direct vicinity of roots leading to formation of rhizoliths, a process which is still not well understood. Stable isotope composition may be a very good proxy for paleoenvironmental reconstructions because of much shorter formation periods compared to other forms of pedogenic carbonates. From the loess-paleosol sequence at Nussloch, SW Germany, rhizoliths were analyzed to

(5) reinforce conclusions drawn from experimental data concerning the timeframe of pedogenic carbonate formation,

(6) evaluate the suitability of these rhizoliths for paleoenvironmental reconstructions with concern to occlusion of older $CaCO_3$ from parent material or postsegregational alteration. This was done based on carbon isotope composition ($\delta^{13}C$, ^{14}C) of $CaCO_3$ and root remains, as well as micromorphological investigations,

(7) elucidate source vegetation of rhizoliths and that of OM in surrounding loess. These results were discussed in the context of previous studies examining source vegetation of rhizomorphic pedogenic carbonates and discussing the chronological context of them in loess-paleosol sequences.

2 Methodological considerations: the isotopic exchange approach

The C isotopic exchange between primary $CaCO_3$ and soil air CO_2 was used to determine amounts of secondary (recrystallized) $CaCO_3$ and consequently $CaCO_3$ recrystallization rates. Therefore, loess containing primary $CaCO_3$ was exposed to CO_2 strongly enriched in a C tracer (^{14}C and, in some of the studies, simultaneously ^{13}C). This labeled CO_2 was either directly added to the loess to assess the effect of CO_2 concentration on the recrystallization rate without interference by biological activity of roots and microorganisms, or released by roots and rhizosphere microorganisms of plants labeled in artificial $^{14}CO_2$ (and $^{13}CO_2$) atmosphere. The strong enrichment of C tracer yielded two advantages: i) isotopic fractionation by CO_2 diffusion in soil and carbonate equilibria reactions (ch. 1.1) was negligible because enrichment was far above natural abundance (NA) of the applied C isotopes, ii) even small amounts (< 0.1 %) of secondary $CaCO_3$ recrystallized during the initial stage of pedogenesis in loess (first weeks – months) could be estimated based on the amounts of incorporated tracer. For calculation of the amounts of recrystallized $CaCO_3$, the

basic assumption of this approach is that the recrystallized portion of loess $CaCO_3$ will inherit the ratio $\frac{\text{C tracer}}{\text{total C}}$ from CO_2 (ch. 2.4).

All labeling experiments (Studies 1 – 5) were performed using loess from Nussloch, SW Germany (Tab. I) for simulation of initial pedogenesis in sedimentary parent material, mainly because of its low C_{org} content (Wiesenberg et al. 2010), high $CaCO_3$ content and primary character of $CaCO_3$ which is fine grained and disseminated homogeneously. Further, loess-derived soils are the most abundant soil types on a global scale.

Table I

Properties of loess from Nussloch, SW Germany, sampled in the open cast mine of the HeidelbergCement AG (N 49°18'41,1'', E 8°43'37,2'').

Year of sampling	depth [m]	$CaCO_3$ [g kg^{-1}]	pH (H_2O)	C_{org} [g kg^{-1}]	WHC [%]
2001	15	290	9	0.1	28
2008	15	274	9	0.3	28

2.1 Labeling procedure without plants

To assess the effect of CO_2 concentration in soil air on the rate of carbonate recrystallization (Study 1), loess samples in metal tubes were directly exposed to $^{14}CO_2$ atmosphere (Fig. IIa). The metal tubes were connected for 10 min to a label solution containing $Na_2{}^{14}CO_3$ and $Na_2{}^{13}CO_3$ from which $^{14}CO_2$ and $^{13}CO_2$ atmosphere were released by addition of H_2SO_4. Increasing amounts of $Na_2{}^{13}CO_3$ in the label solution were applied to obtain three different CO_2 concentrations. After the labeling, the metal tubes were closed until the sampling and subsequent measurement of ^{14}C activity and ^{13}C enrichment.

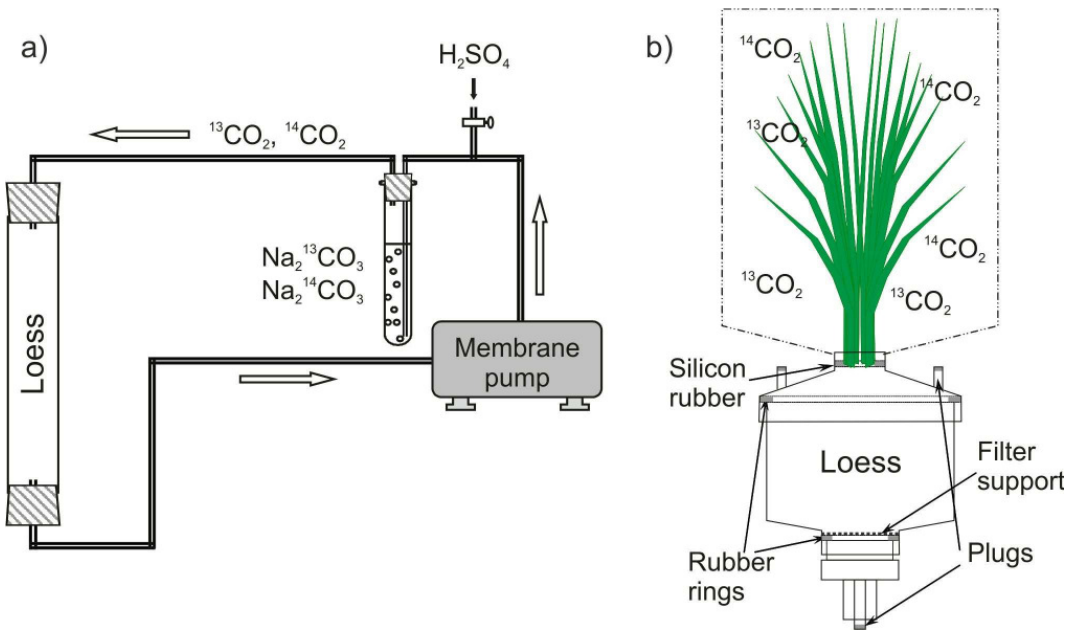

Figure II: Experimental setup. (a) Labeling procedure without plants: ^{14}C and ^{13}C labeled CO_2 is released by addition of H_2SO_4 to the label solution and pumped through the loess sample. (b) Labeling of plants in $^{14}CO_2$ (and $^{13}CO_2$) atmosphere. Sartorius filtration devices were used as loess-root compartment (modified after Kuzyakov and Siniakina 2001).

2.2 Labeling procedure with plants

In studies 2 – 5, the effect of plants and various environmental factors on $CaCO_3$ recrystallization was assessed. Plants were exposed to $^{14}CO_2$ (and $^{13}CO_2$) atmosphere in an airtight chamber for at least 3 hours. $^{14}CO_2$ (and $^{13}CO_2$) were released by addition of H_2SO_4 to the label solution containing $Na_2{}^{14}CO_3$ (and $Na_2{}^{13}CO_3$). The procedure was described in detail by Kuzyakov et al. (2006). Plants were pulse labeled several times and continued growth under normal conditions between the labelings. In contrast to most other ^{14}C labeling studies, rhizosphere CO_2 was not flushed out of the loess-root compartment between the labelings to allow accumulation of root-respired $^{14}CO_2$ and isotopic exchange between the latter and loess $CaCO_3$.

In each experiment and treatment, two kinds of plant pots were used: Pots sealed around the shoot were applied to obtain ^{14}C specific activities from root-respired CO_2 accumulated between labeling and sampling. These specific activities were used to calculate amounts of recrystallized $CaCO_3$ in unsealed pots where CO_2 concentration more likely reflected natural conditions, but ^{14}C specific activities were altered due to air exchange with atmosphere (ch. 2.4).

2.3 Sampling and analyses

At the end of the experiment (Study 1) or in regular intervals after labeling (Studies 2 – 5), the following samples were obtained:

(1) loess (in Studies 2 – 4 divided in rhizosphere and 'root-free' loess),

(2) gaseous CO_2 (in Studies 2 – 5 derived from root and rhizomicrobial respiration), trapped in NaOH to precipitate as Na_2CO_3,

(3) organic and inorganic C dissolved in loess washing water (DOC, DIC; Studies 1 – 5),

(4) solid organic compounds in loess (C_{org}, mainly root fragments; Study 5),

(5) root and shoot biomass (Studies 2 – 5).

In the following, analyses are described only for those pools which are relevant for calculation of recrystallization rates (1, 2).

To determine ^{14}C activity in loess $CaCO_3$, samples were treated with H_3PO_4 to release CO_2 only from loess $CaCO_3$ but not from organic compounds in loess (root fragments, exudates). CO_2 was trapped in NaOH and measured in 6 ml aliquots mixed with 12 ml of scintillation cocktail (Rotiszint, Roth, Germany). ^{14}C activities were determined by a liquid scintillation counter (LSC; either LS 6500 Multi-Purpose Scintillation Counter, Beckman, USA, or 1450 LSC & Luminescence Counter MicroBeta TriLux, Perkin Elmer Inc., USA). ^{14}C activity in root-respired CO_2 was measured similarly. To determine the amount of C in root-respired CO_2, an aliquot of the NaOH was titrated with HCl against Phenolphthalein (Zibilske 1994).

For measurement of $\delta^{13}C$ of loess $CaCO_3$, samples were treated with H_3PO_4 as described above, and CO_2 trapped in NaOH as Na_2CO_3 was precipitated as $SrCO_3$ by addition of $SrCl_2$ solution. The precipitate was purified by centrifugation (Werth and Kuzyakov 2008) and

analyzed by an isotope ratio mass spectrometer (IRMS; Delta Plus XL, Thermo Finnigan, Dreieich, Germany).

2.4 Calculation of recrystallization rates and periods

Calculation of amounts of $CaCO_3$ recrystallized with soil air CO_2 was based on ^{14}C and ^{13}C enrichment in $CaCO_3$. Identical $^{14}C/C_t$ ratio (^{14}C specific activity) and identical $^{13}C/^{12}C$ ratio were assumed for soil air CO_2 and for the portion of recrystallized $CaCO_3$.

For the ^{14}C isotopic approach, amounts of recrystallized $CaCO_3$ were calculated based on ^{14}C specific activity ($^{14}C^{sp}_{CO_2}$) of respired CO_2 in sealed plant pots and ^{14}C activity of loess $CaCO_3$ in open pots ($^{14}C_{CaCO_3}$; Eq. 4).

$$CaCO_{3_{recryst}} = \frac{^{14}C_{CaCO_3}}{^{14}C^{sp}_{CO_2}} \quad (4)$$

Dividing the amount of recrystallized $CaCO_3$ by the amount of total $CaCO_3$ ($CaCO_{3_t}$) and by time (t) between labeling and sampling, the recrystallization rate was determined (Eq. 5).

$$CaCO_3 \text{ recrystallization rate } (^{14}C) = \frac{CaCO_{3_{recryst}}}{CaCO_{3_t} \bullet t} \quad (5)$$

For the ^{13}C labeling approach, $\delta^{13}C$ values from loess $CaCO_3$ were converted into ^{13}C atom percent. Based on ^{13}C mass balance, the recrystallization rate was calculated as ^{13}C atom percent excess in labeled loess carbonate ($A_l^{CaCO_3} - A_{NA}^{CaCO_3}$), divided by atom percent excess in CO_2 respired by ^{13}C labeled plants ($A_l^{CO_2} - A_{NA}^{CO_2}$) and by the time (t) between the labeling and the sampling (Eq. 6).

$$CaCO_3 \text{ recrystallization rate } (^{13}C) = \frac{A_l^{CaCO_3} - A_{NA}^{CaCO_3}}{(A_l^{CO_2} - A_{NA}^{CO_2}) \bullet t} \quad (6)$$

Based on calculated recrystallization rates (RR), time periods necessary for complete recrystallization of loess $CaCO_3$ were determined. As $CaCO_3$ recrystallization is assumed to take place substantially during the growing season when soil CO_2 concentration is high, length of the growing season (GS) was considered for the calculation. Assuming either repeated recrystallization of primary and secondary $CaCO_3$ or one-time recrystallization and subsequent incorporation in growing concretions, amounts of recrystallized $CaCO_3$ will increase either in an exponential or linear way (Kuzyakov et al. 2006; Eqs. 7, 8).

$$CaCO_{3_{recryst}}(t) = 100 \bullet (1 - e^{-t \bullet rate \bullet \frac{GS}{365}}) \quad (7)$$

$$CaCO_{3_{recryst}}(t) = 100 \bullet t \bullet RR \bullet \frac{GS}{365} \quad (8)$$

3 Results and discussion

3.1 Suitability of ^{14}C vs. ^{13}C tracers for accurate quantification of recrystallization (Studies 1, 2)

During the last few decades, the potential of both ^{14}C and ^{13}C tracers has been recognized to elucidate C cycling from plant into soil and within soil by labeling of plants in enriched CO_2 atmosphere (reviewed by Kuzyakov 2001). For assessment of $CaCO_3$ formation rates only ^{14}C has been applied successfully so far (Kuzyakov et al. 2006). Suitability of ^{14}C and ^{13}C tracers was compared by simultaneous labeling of plants in $^{14}CO_2$ and $^{13}CO_2$ atmosphere. This test comprised two different experimental layouts: First, $CaCO_3$ recrystallization rates resulting from direct contact of moistened loess with labeled CO_2 were determined under three different CO_2 concentrations (0.038 – 5 vol.-%) after 4 days (Study 1). Second, recrystallization rates were determined based on tracer enrichment in rhizosphere loess $CaCO_3$ after isotopic exchange with CO_2 respired by plants pulse labeled in artificial $^{14}CO_2$ and $^{13}CO_2$ atmosphere (Study 2).

In particular, study 1 yielded the following findings:

(1) Recrystallization rates based on ^{14}C labeling were successfully determined for all applied CO_2 concentrations and showed values of $0.4 \cdot 10^{-6}$ – $1.7 \cdot 10^{-6}$ day^{-1}, meaning that $n \cdot 10^{-4}$ % of total loess $CaCO_3$ were recrystallized per day. Measurement by LSC resulted in standard errors between replications of < 10 %.

(2) For the ^{13}C labeling approach, rates were one order of magnitude higher ($0.3 \cdot 10^{-5}$ – $1.4 \cdot 10^{-5}$ day^{-1}) and standard errors between replications, resulting from $\delta^{13}C$ analysis by IRMS, were up to > 40 %. For the lowest applied CO_2 concentration (without ^{13}C labeled CO_2) the recrystallization rate could not be determined because the samples did not show significant ^{13}C enrichment when compared to ^{13}C NA of initial untreated loess.

Study 2 yielded the following results:

(3) Based on ^{14}C tracer, the applied plant species (wheat and ryegrass) led to recrystallization rates of $2.9 \cdot 10^{-6}$ day^{-1} and $1.0 \cdot 10^{-6}$ day^{-1}, with standard errors between replications of < 6 %.

(4) The ^{13}C approach provided reasonable values only for the wheat sample set with a rate of $6.3 \cdot 10^{-5}$ day^{-1} and standard error of > 60 %. For ryegrass, which was labeled using half the amount of $Na_2{}^{13}CO_3$ applied for wheat plants, no ^{13}C enrichment was found in loess $CaCO_3$ after labeling. On the contrary, a depletion of up to 0.4 ‰ when compared to ^{13}C NA in loess $CaCO_3$ yielded ‘negative rates’.

These findings led to the conclusion – although results from both ^{14}C and ^{13}C labeling in parts indicated incorporation of C from soil air CO_2 in loess $CaCO_3$ by recrystallization – that the ^{14}C isotopic exchange approach can be recommended for estimation of initial $CaCO_3$ recrystallization rates for the following reasons:

(1) Variations of rates based on the ^{13}C approach equaled or even exceeded the differences between the CO_2 treatments (Study 1) as estimated based on ^{14}C. A highly sensitive

method is required to assess very low amounts of recrystallized $CaCO_3$ after time periods as short as in our experiments, because small uncertainties for the estimated rates lead to huge variations for the modeled time periods for complete recrystallization of loess $CaCO_3$. Precision of the ^{14}C approach was much higher, which was attributed mainly to the lower detection limit of LSC when compared to IRMS (10^{-13} mol vs. 10^{-7} mol), as well as lower background signal from ^{14}C in contrast to ^{13}C NA.

(2) As for ^{13}C, higher precision might be obtained by applying higher amounts of tracer, thus leading to incorporation of higher amounts of ^{13}C in secondary $CaCO_3$. However, this is not recommended because resulting higher CO_2 concentration in the labeling chamber might entail methodological complications (overpressure in the chamber, incomplete tracer assimilation, artificial C partitioning).

(3) Because of the low accuracy of the ^{13}C approach, it was not applied to 'root-free' loess, where even lower recrystallization rates were expected. However, ^{14}C rates were obtained for these loess samples, which i) demonstrated the high reproducibility of the ^{14}C approach by comparison with previous data (Kuzyakov et al. 2006, ch. 3.2.2) and ii) enforced the assumption of decreasing rates with increasing distance to the root surface (ch. 3.2.3).

(4) Based on findings presented in ch. 3.2.2 and 3.2.3, rates should be highest in rhizosphere (Studies 2, 3), decrease towards 'root-free' loess and decrease even stronger for unplanted loess (Study 1). While ^{14}C data followed this trend, ^{13}C data were inconsistent.

Based on these results, ^{14}C was chosen as tracer for all further studies estimating $CaCO_3$ formation and accumulation rates because of its satisfying efficiency. This enabled highest precision while using very low amounts of tracer.

3.2 Effect of environmental factors on $CaCO_3$ recrystallization rates

Intensity and direction of the $CaCO_3 – CO_2 – H_2O$ equilibrium reaction (Eq. 1, ch. 1.1) are driven by pCO_2, pH and concentrations of Ca^{2+} and CO_3^{2-} (Krauskopf and Bird 1995). In a soil profile, these are in turn affected by biological activity of vegetation (root respiration and exudation, metabolites from associated microorganisms) as well as climate (temperature, rainfall) (Arkley 1963, Schlesinger 1985, Birkeland 1999). Effects of these individual environmental factors on $CaCO_3$ recrystallization rates cannot be delineated in the field. They were investigated separately by ^{14}C labeling experiments. In detail, influence of plant species, soil CO_2 concentration, distance from the root surface and temperature were investigated.

3.2.1 Soil CO_2 concentration (Study 1)

The effect of soil CO_2 concentration on $CaCO_3$ recrystallization was elucidated without complication by specific effects of root systems. Therefore, moistened (70 % of WHC) loess samples in closed metal tubes were exposed to differing CO_2 concentration in loess pore space – 380 ppm, 5000 ppm and 50 000 ppm – produced by addition of increasing amounts of ^{13}C labeled Na_2CO_3 to equal ^{14}C activity in each treatment (ch. 2.1). For each treatment,

samples were analyzed 4, 16 and 65 days after the labeling to elucidate the influence of the recrystallization period. Comparison of ^{14}C data brought the following results:

(1) Distribution of recovered ^{14}C between loess $CaCO_3$, DIC in loess washing solution and gaseous CO_2 changed over time, with increasing percentages of ^{14}C in $CaCO_3$ and decreasing recovery in DIC. However, amounts of recrystallized $CaCO_3$ were not significantly different after 4 days compared to longer recrystallization.

(2) Amounts of recrystallized $CaCO_3$ after four days were 10^{-4} % of total loess $CaCO_3$, hence significantly lower than those calculated in plant labeling experiments, although the applied CO_2 concentrations covered approximately the range of natural soil CO_2 concentrations (Davidson 1995). This is probably a result of the one-time CO_2 supply in this study when compared to permanent root respiration during plant growth (Studies 2 – 5), aside from other factors driven by plant growth, e.g. pH (ch. 3.2.3).

(3) Recrystallization rates increased with increasing CO_2 concentrations from $4.1 \cdot 10^{-7} \pm 0.1 \cdot 10^{-7}$ day^{-1} over $8.1 \cdot 10^{-7} \pm 1.7 \cdot 10^{-7}$ day^{-1} to $16.9 \cdot 10^{-7} \pm 1.2 \cdot 10^{-7}$ day^{-1} (Tab. I). According to the Henry's Law, solubility of CO_2 in water is directly proportional to CO_2 partial pressure in equilibrium with water. Therefore, higher CO_2 concentration led to higher amount of CO_2 dissolved in pore water of loess, resulting in dissolution of higher amounts of $CaCO_3$.

(4) Given a constant CO_2 concentration, isotopic reequilibration between gaseous and liquid phase occurs rapidly (sec – min), which resulted in lowest and temporally constant ^{14}C recovery in gaseous CO_2. In contrast, equilibrium between liquid and solid phase is reached after longer time periods but definitely within less than 4 days, because amounts of recrystallized $CaCO_3$ did not change significantly after periods longer than 4 days. This means that the estimated rates give minimum values. Nevertheless, they denote the order of magnitude of $CaCO_3$ recrystallization under the experimental conditions and reveal the dependance of recrystallized $CaCO_3$ on CO_2 concentration.

(5) This relationship reflected a saturation curve, showing steep increase in the range of low CO_2 concentration (atmospheric values - ~ 2 %) and approximating saturation in the range > 3 %.

Based on this obvious relation between soil CO_2 concentration and rates of secondary $CaCO_3$ formation, I hypothesized that vegetation has significant influence on $CaCO_3$ recrystallization in soil, because roots and associated microbial organisms are the main source for soil CO_2 (Amundson et al. 1998). Consequently,

- significant $CaCO_3$ recrystallization should occur mainly during the growing season, when a high CO_2 concentration is maintained by vegetation.
- recrystallization rates in planted soil should exceed those in unplanted soil. Rates should decrease with increasing distance to the root surface because a high CO_2 concentration is maintained only directly on the root surface by permanent CO_2 release, while with increasing distance diffusion increases, entailing strong decrease of CO_2 concentration (Hinsinger et al. 2003; ch. 3.2.3).

- root thickness and distribution as well as rooting density, controlling spatial CO_2 distribution in the soil profile (Hamada and Tanaka 2001), influence $CaCO_3$ recrystallization.

3.2.2 Plant species (Study 3)

Agricultural cereals and pasture plants have different growth rates and distribution of assmilated C (Kuzyakov and Domanski 2000), leading to the assumption that representatives of these two groups might lead to different rates of $CaCO_3$ formation and alteration in soil. To test this hypothesis, wheat [*Triticum aestivum* (L.)] and ryegrass [*Lolium perenne* (L.)] were grown on loess, using Sartorius filtration devices as plant pots (Fig. IIb). Plants received increasing numbers (1 – 5) of ^{14}C pulses (ch. 2.2) in time intervals of 5 days, and amounts of recrystallized $CaCO_3$ in 'root-free' loess were determined 5 days after each labeling (ch. 2.3 and 2.4). The experiment yielded the following findings:

(1) At the initial stage of soil formation (weeks – months) with permanent plant growth, amounts of recrystallized $CaCO_3$ increased linearly, with the slope representing the recrystallization rate.

(2) For wheat and ryegrass, recrystallized $CaCO_3$ in 'root-free' loess amounted to 0.073 ± 0.011 % and 0.072 ± 0.010 % of total loess $CaCO_3$ at day 25 after the first labeling. This translates to recrystallization rates of $3.2 \cdot 10^{-5} \pm 0.2 \cdot 10^{-5}$ day^{-1} and $2.8 \cdot 10^{-5} \pm 0.3 \cdot 10^{-5}$ day^{-1}, respectively (Tab. I).

(3) Despite strongly differing ^{14}C distribution among the pools (ch. 2.3), especially concerning the belowground pools, recrystallization rates did not differ significantly between *Triticum* and *Lolium*.

(4) The amounts of respired CO_2 between two samplings were approximately constant. CO_2 concentrations in plant pots, calculated based on titration of the NaOH with trapped CO_2 (ch. 2.1.3), were not significantly different between wheat and ryegrass with values averaging 6.7 ± 0.5 % and 6.3 ± 0.5 % (v/v). In contrast, amounts of root biomass remained constant during the experiment for wheat but increased for ryegrass from the first to the last sampling date.

From these results the following conclusions were drawn:

(1) The ^{14}C labeling approach is highly reproducible for estimation of $CaCO_3$ recrystallization rates, as shown by comparison of my results with the rate estimated by Kuzyakov et al. (2006; $2.9 \cdot 10^{-5}$ day^{-1}) obtained from a wheat ^{14}C labeling experiment.

(2) ^{14}C distribution patterns reinforced previous studies revealing that the percentage of belowground translocated assimilated C are lower for cereals (Palta and Gregory 1998) and higher for pasture grasses (Kuzyakov et al. 2001).

(3) Higher root biomass and thinner roots of ryegrass vs. wheat provided more surface for rhizosphere processes (respiration, exudation), as reflected by higher percentages of recovered ^{14}C in ryegrass DOC versus wheat DOC. Nevertheless, the expected difference in recrystallization rates under the used plant species could not be shown, presumably because of similar CO_2 concentrations in the loess-root compartment for both treatments.

These CO_2 concentrations were in the range where saturation of carbonate recrystallization occurs, thus leading to similar rates (ch. 3.2.1).

3.2.3 Distance to the root surface (Studies 3, 4)

For experiments performed with Sartorius filtration devices used as plant pots (Fig. IIb), amounts of recrystallized $CaCO_3$ were determined in 'root-free' loess and in rhizosphere loess, the material directly adhering to roots. Based on previous results (ch. 3.2.1), higher rates were expected in rhizosphere loess compared to loess distinct from roots.

Except for ryegrass (Study 3), differences between recrystallization rates in rhizosphere and 'root-free' loess were always significant at the 1 % probability level. $CaCO_3$ recrystallization rates in loess near to the roots were twice (Study 2, performed with wheat and ryegrass) or even 100 times (Study 4, performed with maize; Tab. I) the rate in 'root-free' loess. This wide range of factors might be explained by the sampling design with irregular distinction between rhizosphere and non-rhizosphere material, leading to different portions of 'root-free' loess in analyzed rhizosphere samples. In study 3, this entailed higher standard errors of the mean for calculated amounts of recrystallized $CaCO_3$ in rhizosphere loess and, in case of ryegrass, rather high deviation from the linear trend when compared with 'root-free' loess. Nevertheless, these results clearly demonstrated the influence of living roots on $CaCO_3$ recrystallization in soil. This effect can be attributed to three factors:

(1) High $\underline{pCO_2}$ in pore space, released by permanent root and microbial respiration, leads to increased solubility of CO_2 (as stated by the Henry's Law) which in turn entails decreasing pH because of dissociation of carbonic acid, thus favoring $CaCO_3$ dissolution. Only few studies differentiate CO_2 concentrations in rhizosphere from those in soil distinct to roots, however the effect of root-respired CO_2 is assumed to be locally limited (Hinsinger et al. 2003) because of higher diffusivity of CO_2 when compared to soil solution containing DIC. As a consequence, $CaCO_3$ dissolution and recrystallization should be higher in rhizosphere loess than in 'root-free' loess, which was confirmed by my results.

(2) $CaCO_3$ dissolution and reprecipitation are further influenced by <u>pH</u>. The pH of a soil solution, especially in rhizosphere, is affected by several factors (Hinsinger et al. 2003). Besides CO_2 release by root respiration (see above), the following factors were most relevant with regard to pH in the here presented study.

- Bulk soil pH and pH buffering capacity: High $CaCO_3$ content and thus high pH of the applied loess (Tab. I) buffers pH variations in 'root-free' loess. However, the effect of increased root and rhizomicrobial respiration on rhizosphere pH is much higher in calcareous than in acidic soils (Gras 1974, Nye 1981), leading to a steep pH gradient from the root surface towards 'root-free' loess.
- Root exudation: Release of organic anions from fatty acids, as well as H^+ or OH^- for neutral anion-cation balance, influence pH and therefore $CaCO_3$ recrystallization. The anion-cation balance might have been influenced by providing the plants with a

modified version of Hoagland nutrient solution (Hoagland and Arnon 1950) that contained nitrogen (N) solely as NO_3^- in rather large quantity.

Regarding the fact that CO_2 flux from root and microbial respiration considerably exceeds H^+ or OH^- release (Durand et al. 2001), high CO_2 concentration has still major influence on advance of $CaCO_3$ recrystallization.

(3) While CO_2 concentration and pH affect mainly $CaCO_3$ recrystallization in direct vicinity of the roots (few mm), transport of dissolved Ca^{2+} and DIC by mass flow might lead to enhanced secondary $CaCO_3$ precipitation in a diameter of up to several cm around the roots. In calcareous sediments, water transport resulting from transpirational pull of the plant finally may lead to formation of rhizoliths (Klappa 1980) within longer time periods than in the presented study (years to centuries).

As stated above, assessment of the extent of the rhizosphere effect on $CaCO_3$ recrystallization was precluded by the experimental design. Based on considerations about rhizosphere CO_2, I hypothesize highest recrystallization rates at the root surface and a strong decrease within the first few mm from the root surface towards 'root-free' loess.

3.2.4 **Temperature** (Study 4)

The equilibrium reaction between $CaCO_3$, CO_2 and H_2O (Eq. 1) is strongly influenced by temperature: On one hand, solubility of CO_2 and, to a smaller extent, of $CaCO_3$ in pure water decreases with increasing temperature, thus diminished dissolution of loess carbonate might be expected under high temperature. On the other hand, in natural waters, lower CO_2 concentration and therefore higher pH at high temperature leads to stronger precipitation of dissolved $CaCO_3$ under high temperatures (Krauskopf and Bird 1995). Moreover, biological processes like plant growth, root respiration, exudation and microbial respiration are promoted by increasing temperature. Thus, enhanced CO_2 production should influence $CaCO_3$ recrystallization in the opposite way. To test this hypothesis, maize plants were grown at three different temperatures of 10, 20 and 30 °C, with equal moisture conditions in each treatment (70 % of WHC). Application of 1 – 4 ^{14}C isotopic pulses (ch. 2.2) and subsequent ^{14}C analyses (ch. 2.3) revealed lowest $CaCO_3$ recrystallization rates at lowest temperature and highest rates at highest temperature. In 'root-free' loess, temperatures of 10, 20 and 30 °C led to rates of $0.29 \cdot 10^{-5} \pm 0.03 \cdot 10^{-5}$ day^{-1}, $0.57 \cdot 10^{-5} \pm 0.07 \cdot 10^{-5}$ day^{-1} and $3.10 \cdot 10^{-5} \pm 0.40 \cdot 10^{-5}$ day^{-1} (Tab. I). These results clearly showed that temperature-dependant solubility of CO_2 and $CaCO_3$ are negligible. In contrast, temperature has an enormous indirect influence on initial $CaCO_3$ recrystallization rates via enhancement of biological activities. Possible effects of biogenic processes on pedogenic carbonate formation and accumulation have long been ignored (Goudie 1996). The promoting effect of temperature on recrystallization rates can be explained to some degree by increasing CO_2 production: CO_2 concentrations in the loess-root compartment after accumulation during five days (averaged from four samplings) increased from 2.2 ± 0.3 % at 10 °C to 5.8 ± 0.6 % at 20 °C, but were not significantly different for the latter and the 30 °C treatment (6.0 ± 0.5 %). Therefore, another factor must be responsible for the major increase of $CaCO_3$ recrystallization rates between 20 °C and 30 °C. As a consequence of increasing water uptake by plants with increasing temperature, moisture

levels were rather constant at 10 °C (minimum 65 % of WHC) and varied strongest at 30 °C (minimum 25 % of WHC) despite daily adjustment. Transpiration probably affects suction pressure in soil stronger than evaporation (Klappa 1983). Strongly contrasting moisture conditions are thought to promote pedogenic $CaCO_3$ precipitation (Becze-Deák et al. 1997), because each drying increases ion concentrations, finally leading to saturation of the soil solution with respect to Ca^{2+} and HCO_3^- and precipitation as $CaCO_3$ (Birkeland 1999). Hence, strongest evapotranspiration and consequently strong contrast of moisture level at 30 °C when compared to the other two treatments promoted precipitation of secondary $CaCO_3$ not only in rhizosphere but even in 'root-free' loess.

This study showed for soils covered by higher plants, that $CaCO_3$ recrystallization takes place faster under warm climates compared to colder climates, as long as regions with equal amounts of rainfall are compared. Further influence of temperature on $CaCO_3$ recrystallization periods is discussed in ch. 3.3.

3.3 Recrystallization periods (Studies 1, 3, 4)

Table II
$CaCO_3$ recrystallization rates for unplanted (Study 1) and planted loess (Studies 3, 4) at various conditions.

recryst-allization rates		Study 1			Study 3		Study 4 (*Zea maize*)		
Factor		CO_2			plant species		temperature		
		380 ppm	5000 ppm	50 000 ppm	*Triticum aestivum*	*Lolium perenne*	10 °C	20 °C	30 °C
[10^{-7} day^{-1}]	unplanted loess	4.1 [± 0.1]	8.1 [± 1.7]	16.9 [± 1.2]					
[10^{-5} day^{-1}]	'root-free' loess				3.2 [± 0.2]	2.8 [± 0.3]	0.29 [± 0.03]	0.57 [± 0.07]	3.10 [± 0.40]
	rhizosphere loess				6.8	4.7	20.6 [± 2.0]	27.8 [± 5.7]	74.6 [± 8.7]

$CaCO_3$ recrystallization rates obtained from various experiments were in the range of 10^{-7} day^{-1} (Study 1) in unplanted loess, and mostly two orders of magnitude higher (10^{-5} day^{-1}; Studies 3, 4) when CO_2 was permanently released by roots (Tab. II). These rates were used to estimate periods necessary for recrystallization of loess $CaCO_3$ (Eq. 7, 8; ch. 2.4). In the following, if not indicated otherwise, amount of secondary $CaCO_3$ is assumed to increase in an exponential way. In general 95 % recrystallization of total loess $CaCO_3$ was regarded, because a remaining portion of ≤ 5 % of primary $CaCO_3$ is too small to be detected by IRMS analysis of ^{13}C NA. For experiments with planted loess, length of growing season was accounted for, because high CO_2 concentration in soil, caused by root and rhizomicrobial

respiration, leads to considerably faster recrystallization during that time when compared to conditions without permanent CO_2 supply (ch. 3.2.1). Obviously, a short growing season strongly increases the recrystallization period and *vice versa*. Extrapolation of the estimated rates yielded the following results:

(1) In unplanted loess, rates represented the 'background' recrystallization without vegetation, as is the case e.g. in soils of extreme desert. Assuming this process to occur during 365 days of the year, complete recrystallization occurred within 20 000, 10 080 or 4860 years for CO_2 concentrations of 380, 5000 or 50 000 ppm, respectively. These periods have to be regarded as maximum under the given conditions because rates were obtained after 4 days, whereas equilibrium between $CaCO_3$, CO_2 and H_2O might have occurred earlier (ch. 3.2.1), possibly after 2 – 3 days. Nevertheless, they point out the order of magnitude for recrystallization periods which are in the range of 10^3 – 10^4 years.

(2) In contrast to unplanted loess, shorter periods were calculated for loess distant to roots ('root-free' loess). Using typical growing seasons of 4 and 6 months for wheat and ryegrass (Study 3), corresponding recrystallization periods of 800 and 600 years were calculated. However, growing seasons can be considerably shorter under arid and semiarid climatic conditions, typical for secondary carbonate formation. Applying a growing season of only 2 months yielded significantly longer recrystallization times of 1600 and 1800 years for wheat and ryegrass, respectively (Fig. IIIa). As for maize (grown at 20 °C; Study 4), complete recrystallization would take 8700 years with a growing season of 2 months, but only 2900 years at 6 months. These results indicate that in grassland soils complete recrystallization is reached within 10^2 – 10^3 years. Comparing these periods with those of unplanted loess shows the enormous meaning of vegetation for secondary $CaCO_3$ formation and recrystallization in soil.

(3) In rhizosphere, $CaCO_3$ recrystallization rates were higher when compared to 'root-free' loess (ch. 3.2.3). First, it might be assumed that recrystallization occurs similarly adjacent to and distant from roots, i.e. repeated recrystallization of primary and secondary $CaCO_3$. With a growing season of 4 months, 95 % recrystallization of total loess $CaCO_3$ would be completed after 360 years in case of wheat (Study 3), which is considerably shorter compared to 800 years in 'root-free' loess. However, conditions favoring $CaCO_3$ recrystallization in vicinity of grass roots (ch. 3.2.3) are not supported for longer time because of short-term spatial modification of the root system. On the other hand, accretion of secondary carbonate around roots, i.e. rhizolith formation (ch. 3.5) results in even shorter periods, because a linear increase of secondary $CaCO_3$ can be assumed if growing $CaCO_3$ crystals impede further dissolution and reprecipitation of secondary carbonate. If assuming evergreen plants as pioneer vegetation on loess with growing season of up to 8 months, the time necessary for 95 % recrystallization is less than 60 years (Fig. IIIa). This period, extrapolated with rhizosphere recrystallization rates of wheat, is just an approximation because initial recrystallization rates of woody plants, the presumed source vegetation of rhizoliths (ch. 3.5) is unknown. Nevertheless, these findings agree with the assumption that rhizoliths form rather fast, within 10^1 – 10^2 years at maximum. Previous

studies suggested rhizolith formation during the plant's life time and/or beginning decay (Wang and Greenberg 2007, Klappa 1980). Short formation times of rhizoliths make this type of secondary carbonate a useful tool for paleoenvironmental reconstructions based on $\delta^{13}C_{carb}$ (e.g. Pustovoytov and Terhorst 2004, Wang and Greenberg 2007) because they provide high methodological resolution of these studies.

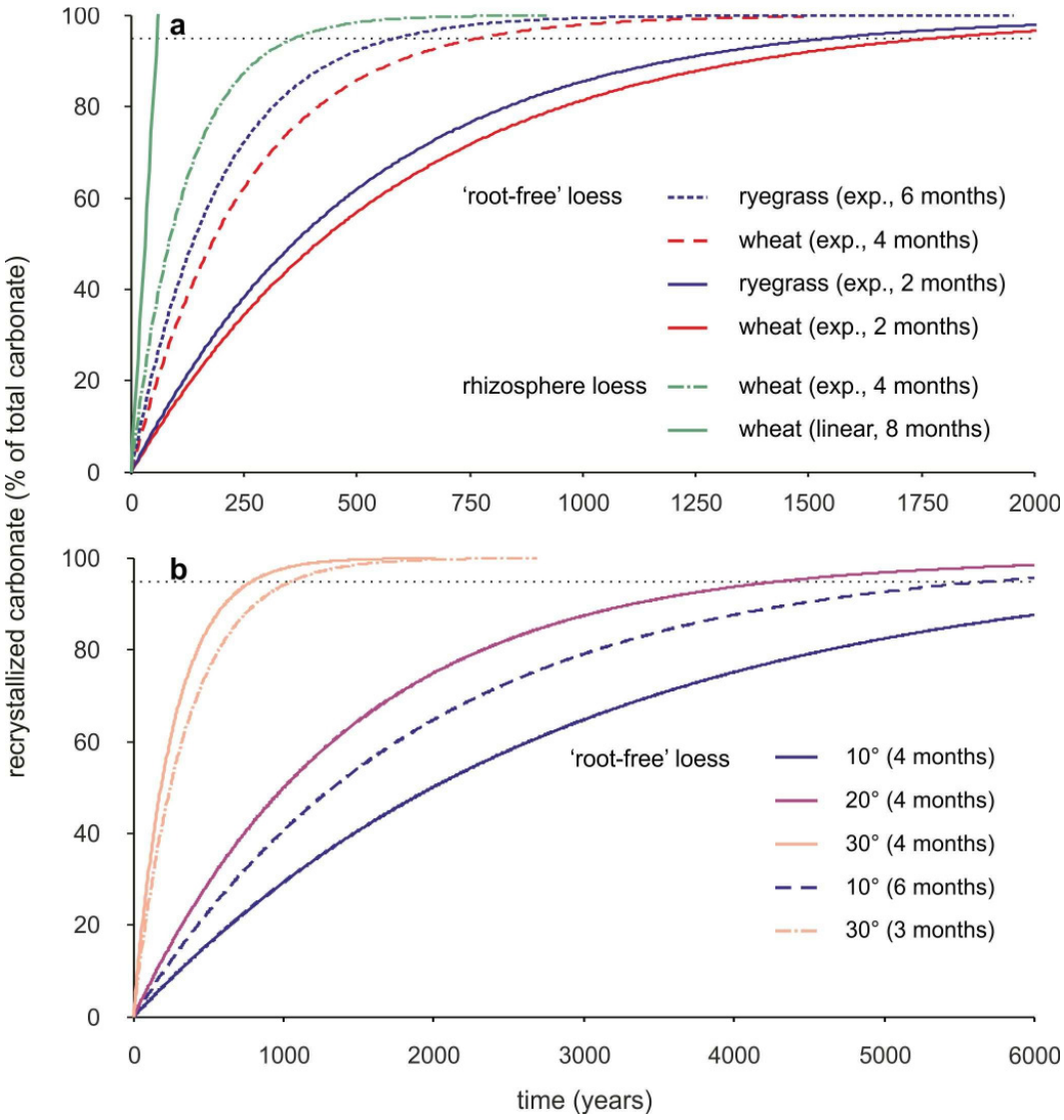

Figure III: Comparison of $CaCO_3$ recrystallization periods extrapolated from recrystallization rates. (a) Periods necessary for complete recrystallization of total loess $CaCO_3$ in rhizosphere and 'root-free' loess. (b) Periods necessary for complete recrystallization of total loess $CaCO_3$ at 10, 20 and 30 °C as depending on length of growing season (given in parentheses). Black dashed lines represent the level of 95 % recrystallization.

(4) Temperature strongly influences pedogenic carbonate recrystallization rates (ch. 3.2.4). This is reflected in shorter recrystallization periods under higher temperatures: 95 % of loess $CaCO_3$ would be recrystallized after 8610, 4330 or 800 years at 10, 20 or 30 °C if equal growing seasons of 4 months are assumed. However, at constant amount of rainfall, increasing temperature leads to decreasing length of the growing season because of decreasing amounts of soil water available to the roots. This somewhat compensates the positive relation between temperature and recrystallization rates. Assuming e.g. growing

seasons of 6, 4 and 3 months at 10, 20 and 30 °C, $CaCO_3$ recrystallization would take 5740 years, 4330 years and 1060 years (Fig. IIIb).

Among the various types of pedogenic carbonates (ch. 1.1), those formed distant from roots (e.g. coatings, nodules) are most frequently used for paleoenvironmental reconstructions. For these, extrapolation of non-rhizosphere recrystallization rates reflects best the time frame of their formation. It should be considered that recrystallization periods obtained from ^{14}C labeling experiments represent minimum times under the given conditions. Rates were overestimated because of high CO_2 concentrations in the loess-root compartment and rather humid conditions. Additionally, in soils of arid regions, lower rooting density and rooting depth might cause lower recrystallization rates. Despite that, the estimated periods indicate the temporal order of magnitude of pedogenic carbonate formation in calcareous sedimentary parent material. The conclusion that pedogenic carbonates distant from roots form and recrystallize mostly within thousands of years is supported by previous studies based on different methodological approaches: radiocarbon dating (Chen and Polach 1986, Pustovoytov et al. 2007), combined $\delta^{13}C$ analyses and radiocarbon dating (Pendall et al. 1994) or ^{14}C pulse labeling under controlled conditions (Kuzyakov et al. 2006). As mentioned in ch. 1.2, these estimated periods for secondary $CaCO_3$ formation and recrystallization reflect the limit for the methodological resolution of paleoenvironmental reconstructions based on pedogenic carbonate $\delta^{13}C$.

3.4 Pedogenic carbonate formation: recrystallization vs. migration (Study 5)

Depth and rate of carbonate accumulation increase with increasing amounts of rainfall (Marion 1989, Birkeland 1999) and are used in paleosols as proxy for paleoprecipitation (reviewed by Sheldon and Tabor 2009) and determination of soil age (Gile 1993). These rates were obtained from mature soil profiles developed over long periods (10 000s of years) and mostly on non-calcareous parent material. The aim of our study was to show the depth-related redistribution of secondary $CaCO_3$ during initial pedogenesis in a calcareous parent material (loess) in an experiment with alternating moisture conditions, and to estimate periods necessary for decalcification of upper layers.

Maize plants were grown on loess-filled columns (height 85 cm, diameter 43 mm) and pulse labeled in $^{14}CO_2$ atmosphere in time intervals of 3 weeks. During plant growth, moisture conditions in the uppermost 60 cm of loess were alternated between 30 and 70 % of WHC by gravimetric adjustment. Water was added 21 – 22 hours after beginning of each labeling because maize roots release the major part of recently assimilated CO_2 during the first hours after assimilation (Kuzyakov and Cheng 2004). Thereafter, moisture level was readjusted to 70 % when it had dropped below 35 % by plant transpiration. After six months, amounts of recrystallized $CaCO_3$ were determined, based on ^{14}C isotopic exchange, in 5 cm slices from top to bottom of the columns.

As expected after this short period of initial plant growth and pedogenesis, possible mass changes of total $CaCO_3$ due to redistribution were too low for detection. In contrast, based on ^{14}C isotopic exchange between root-respired CO_2 and $CaCO_3$, significant redistribution of

recrystallized $CaCO_3$ was demonstrated, with highest amounts (> 0.3 % of total loess $CaCO_3$) between 20 and 35 cm and lowest amounts (< 0.1 %) in the uppermost 10 cm as well as below 45 cm depth (Fig. IVb). As suggested by previous findings relating recrystallization rates to CO_2 concentration (ch. 3.2.1), amounts of recrystallized $CaCO_3$ should be approximately proportional to root biomass if regarding one plant species. Loess columns were, however, completely rooted (Fig. IVa) and high CO_2 respiration rates from fine root hairs likely prevailed also in deeper parts of the loess column. Consequently, the distribution pattern of recrystallized $CaCO_3$ results from migration by percolating water. Total amount of recrystallized $CaCO_3$ (534 ± 28 mg of loess $CaCO_3$, corresponding to 0.12 ± 0.01 %) and root biomass in each slice were used to calculate theoretical amounts of recrystallized $CaCO_3$. Fig. IVb shows theoretical secondary $CaCO_3$ distribution, assuming that no migration of recrystallized $CaCO_3$ had occurred and each $CaCO_3$ molecule was dissolved and reprecipitated in equal depth. Comparison of calculated and theoretical amounts of secondary $CaCO_3$ showed that downward transport of dissolved Ca^{2+} and HCO_3^- resulted in loss of 81.5 ± 3.2 % of secondary carbonate in the uppermost 15 cm. A secondary $CaCO_3$ accumulation zone, formed between 15 and 50 cm, comprised 72.6 ± 4.5 % of total recrystallized $CaCO_3$ (Fig. IVc). As water movement in loessic soils is rather fast (usually 10 – 100 cm day^{-1}), migration is supposed to result not directly from percolating water but from displacement of the solution (30 % of WHC) saturated with respect to Ca^{2+} and HCO_3^- by the next water addition. Oversaturation and subsequent reprecipitation were caused by decreasing amounts of percolating water with increasing depth (Arkley 1963) and increasing water uptake by roots (Schlesinger 1985). Lacking carbonate accumulation in the uppermost 15 cm suggested that the rate of $CaCO_3$ migration, resulting from downward transport of dissolved Ca^{2+} and HCO_3^-, is higher than the rate of $CaCO_3$ recrystallization by C exchange between carbonate and root-respired CO_2. Consequently, the rate at which a carbonate accumulation horizon forms in loess is limited by the $CaCO_3$ recrystallization rate rather than by the rate of its downward transport. Hence, the theoretical rate of uppermost 15 cm ($1.77 \cdot 10^{-5} \pm 0.26 \cdot 10^{-5}$ day^{-1}) was used to estimate the period necessary for 95 % decalcification of this loess layer. Assuming a linear decrease of $CaCO_3$ content by one-time dissolution and removal (Eq. 8), 300 – 1760 years were calculated applying growing seasons of 6 – 1 months. These results, obtained under rather high moisture conditions, agree with decalcification periods for calcareous parent materials in humid regions like NW Europe (Jenny 1994). In contrast, accumulation of secondary $CaCO_3$ and formation of a calcic horizon in deeper horizons involves repeated dissolution and reprecipitation of primary and secondary $CaCO_3$ (Eq. 7), leading to longer periods. Keeping this in mind, our modelled data fit in terms of magnitude with field data for pedogenic carbonate accumulation periods in calcareous parent material (e.g. 3.5 – 5 kyears in the Northern Caucasus; Alexandrovskiy 2000).

This study showed that a calcic horizon in loess is formed by migration of solely recrystallized $CaCO_3$. Therefore, isotopic signature of $CaCO_3$ can be used for paleoenvironmental reconstruction. However, the precision of such reconstructions cannot be better than the periods necessary for calcic horizon formation.

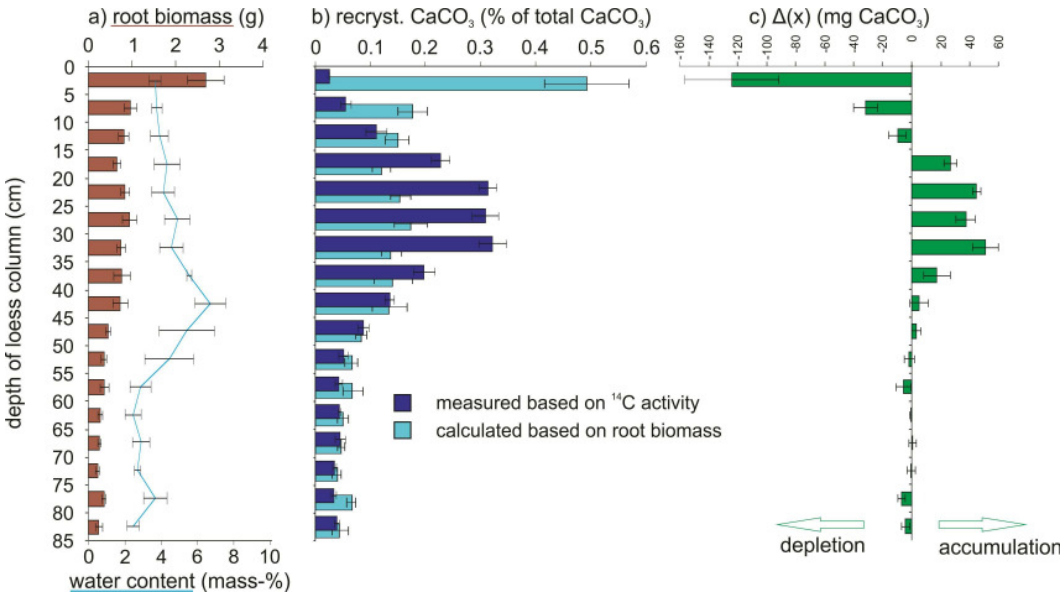

Figure IV: Depth-dependant distribution of a) amounts of root biomass and loess moisture measured at the end of the experiment, b) amounts of measured and theoretically recrystallized amounts of $CaCO_3$, assuming no downward translocation (see Equation 8), c) loss (left) and accumulation (right) of secondary $CaCO_3$. Mean values ± SEM, n = 5.

3.5 Rhizoliths in loess: implications for paleoenvironmental studies

Model data demonstrated the meaning of vegetation for the formation of pedogenic carbonates (Cerling 1984, Nordt et al. 1996). This relation becomes evident when regarding rhizomorphic carbonate concretions like calcified root cells (Pustovoytov and Terhorst 2004), rhizoliths (Klappa 1980) and rhizogenic calcrete (Alonso-Zarza 1999, Alonso-Zarza and Arenas 2004). Precipitation of secondary $CaCO_3$ around roots, leading to rhizolith formation, is still not well understood. Rhizoliths have been often described in literature (Ziehen 1980 and references therein) and are thought to be a valuable paleoenvironmental indicator (Becze-Deák et al. 1997). However, potential incorporation of C of other age may complicate interpretation of paleoenvironmental and chronological information. Inheritance of older carbonate from parent material leads to overestimated radiocarbon ages, whereas introduction of younger C by postsegretational alteration entails underestimated ^{14}C ages (Chen and Polach 1986, Amundson et al. 1994, Budd et al. 2002). Further, following questions concerning rhizoliths have not been clarified so far: Are rhizoliths the result of fossilization, i.e. encrustation of decaying roots (e.g. Joseph and Thrivikramaji 2006), or is rhizolith formation an active process, performed by living roots e.g. for water uptake? Assuming the latter, which plants are able to induce rhizolith formation? Did rhizoliths in sedimentary environment form synsedimentary, as supposed by most authors (e.g. Becze-Deák et al. 1997, Wang et al. 2000, 2004), or postsedimentary, a possibility which was recognized only scarce (Pustovoytov and Terhorst 2004)?

Rhizoliths and surrounding loess from the late Pleistocene loess-paleosol sequence at Nussloch, SW Germany, were investigated to elucidate their formation in chronological context, suitability of their C isotope composition for paleoenvironmental studies and their source vegetation. Most of the loess at Nussloch was deposited during the last glacial-

interglacial cycle (Zöller et al. 1988; Hatté et al. 1998) with termination of loess sedimentation 15 kyears B.P. (Antoine et al. 2001). In the investigated profile, rhizoliths occurred locally abundant with approximately 10 – 20 rhizoliths m^{-2}.

3.5.1 Carbon isotope composition and micromorphology (Study 6)

In depths between 1 and 8 m below present surface, distinct hard rhizoliths of up to 5 cm in diameter occur predominantly in vertical orientation within the profile, indicating that they formed *in situ*. Their size, together with the fact that some individual rhizoliths can be traced downward the profile over more than 1 m, suggested higher plants as source vegetation.

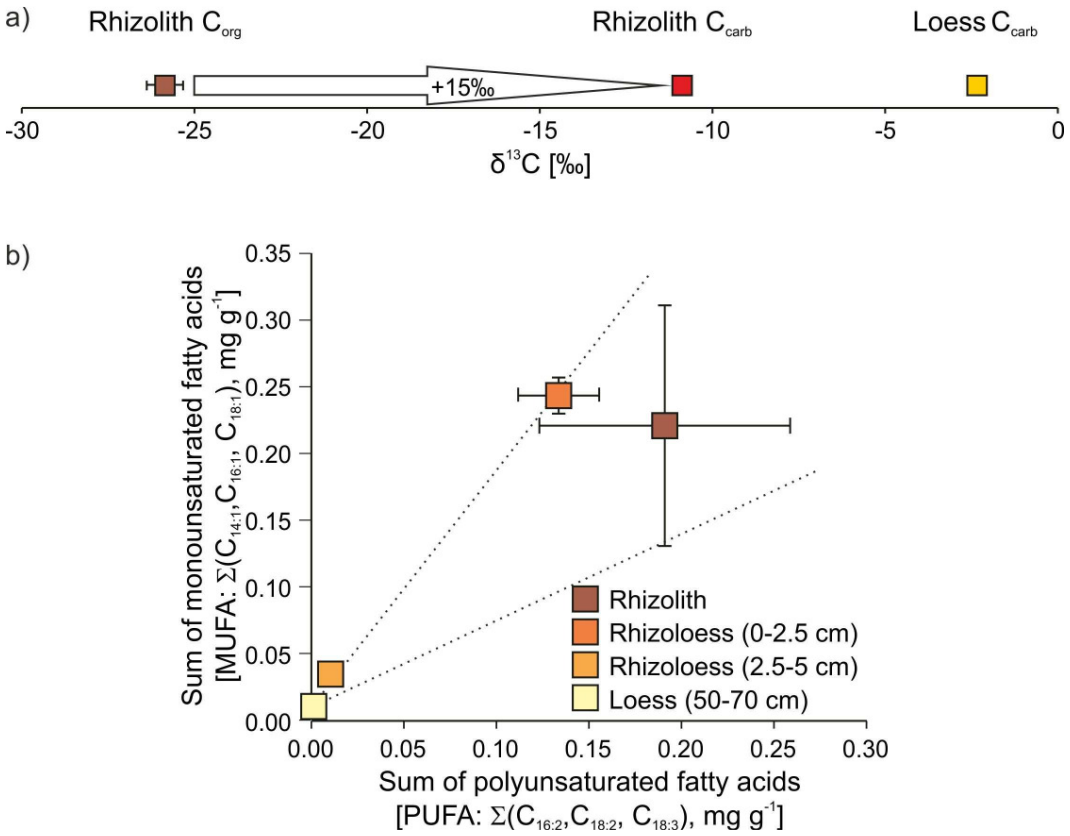

Figure V: Stable C isotopic and lipid composition of rhizoliths and loess. (a) Average $\delta^{13}C_{carb}$ values from loess (reference loess 1 from 15 m below present surface, reference loess 2 from 2.2–2.6 m below present surface) and rhizoliths, and $\delta^{13}C_{org}$ values from rhizoliths. (b) Abundances of MUFAs and PUFAs in rhizoliths, rhizoloess and loess, normalized to total lipid contents (mg g^{-1} extract). Dashed lines represent area of mixing between loess OM and rhizolith OM. Values outside this zone indicate a significant accumulation of additional microbial biomass and degradation products, especially in RL1.

Using $\delta^{13}C$ of rhizolith $CaCO_3$ (–10.9 ± 0.1 ‰) as well as that of organic remains (–25.9 ± 0.5 ‰; Fig. Va) found within rhizoliths from a depth between 1 and 3 m, source vegetation of the Nussloch rhizoliths was identified as plants following the C_3 photosynthetic pathway (Nordt et al. 1996). Portions of secondary $CaCO_3$ in rhizolith carbonate were calculated based on mass balance (Nordt et al. 1998) using $\delta^{13}C$ values of primary loess $CaCO_3$ (–2.4 ± 0.1 ‰), rhizolith carbonate and rhizolith OM. High portions of secondary $CaCO_3$ (98.1 ± 1.1 %) indicated that rhizolith carbonate was almost free from enclosed primary loess $CaCO_3$, thus enabling measurement of reliable radiocarbon ages. These results,

in combination with Holocene radiocarbon ages of $CaCO_3$ (3788 ± 59 years B.P.) and OM (3150 ± 59 years B.P.) of one rhizolith sample from a depth of 1.3 m, led to the following conclusions:

(1) CO_2 released by root and rhizomicrobial respiration was the main or sole source for rhizolith $CaCO_3$, as indicated by $\Delta^{13}C_{CaCO_3\text{-}OM} = 15$ ‰ (Cerling 1984, Cerling et al. 1989).

(2) Roots were calcified during their lifetime.

(3) Carbonate of the rhizoliths was not appreciably contaminated by younger C related to postsegregational alteration.

(4) Despite presumably fast formation (ch. 3.3), very close ^{14}C ages of rhizolith $CaCO_3$ and OM suggest that rhizoliths do not contain considerable amounts of primary $CaCO_3$. The difference between measured ^{14}C ages ($\Delta \approx 640$ years) yielded a portion of primary carbonate of 7.4 % which might be attributed to occlusion of less soluble dolomite ($CaMg[CO_3]_2$). In contrast to earlier studies referring to the 'limestone dilution effect' (Williams and Polach 1971), it is currently believed that overestimated radiocarbon ages of pedogenic carbonates are caused either by mechanical admixture of primary carbonate (Amundson et al. 1989, Monger et al. 1998) or by microbial decomposition of ^{14}C-depleted OM (Wang et al. 1994). The former seems more likely, because presence of few sparitic (primary, i.e. stronger ^{13}C enriched) carbonate grains in the outer part of some of the rhizoliths was confirmed by micromorphological analyses of cross sections, as well as by slightly increasing $\delta^{13}C_{carb}$ values from the center to the outer margin of the rhizoliths.

The radiocarbon age of the analyzed samples did not allow to draw general conclusions regarding the time span and thus climatic conditions promoting rhizolith formation (Ziehen 1980). However, this study showed that loess-paleosol sequences can be penetrated postsedimentary by deep-rooting plants other than the source vegetation of synsedimentary incorporated OM. Presumably, considerable amounts of exudates released by these roots during their life time (Kuzyakov and Domanski 2000, Nguyen 2003) led to incorporation of significant amounts of younger OM and potential overprint of the original isotopic signal ($\delta^{13}C_{org}$, ^{14}C age) of synsedimentary loess OM (LOM) in the vicinity of roots. Differentiation between rhizosphere loess (from the rhizolith surface up to 5 cm distance) and reference loess (50 – 70 cm distant from rhizolith) was not possible by C_{org} and C_{carb} contents. Additionally, $\delta^{13}C_{org}$ of rhizosphere and reference loess did not reveal this possible incorporation of postsedimentary OM either, because both loess OM and rhizolith OM derive from C_3 vegetation (Hatté et al. 1998, own results; Fig. Va). However, portions of secondary $CaCO_3$ in rhizosphere loess of up to 12 % strongly indicated an effect of pedogenic processes (recrystallization) in the former rhizosphere.

3.5.2 Lipid composition of root remains and loess OM (Study 7)

To obtain information about source vegetation of rhizoliths and that of synsedimentary loess OM, lipid composition of both materials was compared. Based on lipid molecular proxies, transects from the rhizolith (R) via rhizosphere loess (RL) towards reference loess (L) were used for estimation of the input of root-derived postsedimentary OM in loess. Lipids,

which are ubiquitous in living tissues, account substantially for OM in plant biomass and SOM and provide several diagnostic markers for source apportionment (Wiesenberg et al. 2004a). Two lipid fractions, fatty acids (FA) and alkanes, were obtained from rhizolith and loess OM by Soxhlet extraction and SPE separation (Wiesenberg et al. 2004b, 2010), and analyzed via GC-FID. Based on FA and alkane composition and molecular proxies, several sources of OM were determined with the following conclusions:

(1) OM of R and L reflected mainly plant biomass. Differences concerning the most abundant long chain FA (LCFA) and alkanes in R and L indicated different source vegetation. The most abundant long chain alkane (LCA) in loess, *n*-C_{31}, further suggested grass as source vegetation, while the most abundant LCA in some of the rhizoliths, *n*-C_{29}, is not specific for grass or woody vegetation, respectively (Maffei 1996a, b).

(2) High amounts of LCFA (C_{22-32}) as well as highest C_{org}-normalized lipid contents in loess OM compared to RL and L indicated its origin from above- and belowground plant biomass, thus reflected steppe conditions with predominantly grass vegetation (pers. comm. G. Wiesenberg). This is in agreement with the general assumption of loess deposition taking place during glacial times with scarce vegetation cover (Bai et al. 2009).

(3) The carbon preference index (CPI) of LCFA and LCA is an indicator of the degree of OM degradation, with high values in fresh plant biomass, while degradation and microbial reworking lead to low values (Peters et al. 2005). Analogous, average chain length (ACL) of FA and alkanes are used to distinguish between plant biomass (high values) and OM deriving from microbial biomass or mixed plant and microbial sources (low values; Harwood and Russell 1984). In RL, lowest CPI and ACL for both FA and alkanes indicated that this loess was strongly affected by rhizosphere processes including incorporation of root remains and microbial degradation of roots and old (synsedimentary) OM. This agrees with the hot-spot theory, attributing high microbial activity and the presence of large amounts of root fragments, exudates and microbial metabolites to the rhizosphere (Jones 1998).

(4) Further, the presence of plant- and microorganism-derived monounsaturated FA (MUFA, $C_{14:1}$, $C_{16:1}$, $C_{18:1}$) and plant-derived, readily degradable polyunsaturated FA (PUFA, $C_{16:2}$, $C_{18:2}$, $C_{18:3}$) in R and RL and their very low abundance or absence in L clearly showed the comparatively severe degradation of LOM (Fig. Vb). Increasing amounts of MUFA and PUFA from L via RL towards R showed the notable contribution of less degraded, potentially younger OM, related to former roots, to LOM in rhizosphere. Assuming R and L as endmembers with 100 % and 0 % root-derived OM, portions of root- and microbial-derived OM were calculated based on MUFA and PUFA as 11.5 % and 4.9 % in a distance of 2.5 – 5 cm from the rhizolith.

In Quaternary terrestrial sediments with high chronological resolution like loess, $\delta^{13}C_{org}$ is used as a proxy for paleovegetation and -precipitation (e.g. Hatté and Guiot 2005). The possibility of postsedimentary incorporation of biomass in terrestrial sediments, potentially leading to considerable uncertainties concerning paleoenvironmental studies, has been recognized by few authors (Head et al. 1989, Zhou et al. 2005). This study showed that

incorporation of root- and microbial-derived OM can overprint synsedimentary LOM and consequently its ^{13}C and ^{14}C isotopic signature as well as molecular composition at least to a distance of 5 cm from former roots and presumably even in greater distances. Further studies are necessary for an exact quantification of this potential overprint which may be used to correct the measured isotopic signature of LOM.

4 Conclusions and outlook

The isotopic exchange approach was shown to be a useful tool to assess slow rates of pedogenic (secondary) $CaCO_3$ formation under controlled conditions. So far, this is the only method for successful estimation of $CaCO_3$ recrystallization rates. Application of ^{14}C as isotopic tracer was preferred over ^{13}C because of higher precision of the former, resulting mainly from a lower detection limit of ^{14}C (by LSC) vs. ^{13}C measurement (by IRMS) and the absence of a background signal for ^{14}C in terms of the used instrument. Subsequently, amounts of recrystallized $CaCO_3$ in loess were calculated based on quantification of ^{14}C tracer incorporated from artificially labeled $^{14}CO_2$.

Within the range of naturally occurring soil CO_2 concentrations, $CaCO_3$ recrystallization rates increased with ascending CO_2 concentration from $4.1 \cdot 10^{-7}$ day^{-1} to $16.9 \cdot 10^{-7}$ day^{-1}. This indicated a strong effect of soil CO_2 concentration on $CaCO_3$ recrystallization rates and suggested highest rates in rhizosphere, where CO_2 concentration is permanently high due to root and rhizomicrobial respiration. ^{14}C labeling of plants and subsequent quantification of ^{14}C tracer incorporated in secondary $CaCO_3$ from root- and microbial-respired CO_2 confirmed this assumption: Independent of plant species, significantly higher recrystallization rates occurred in rhizosphere loess vs. 'root-free' loess (e.g. wheat $6.8 \cdot 10^{-5}$ day^{-1} vs. $3.2 \cdot 10^{-5}$ day^{-1}, maize $27.8 \cdot 10^{-5}$ day^{-1} vs. $0.6 \cdot 10^{-5}$ day^{-1}). This demonstrated the importance of vegetation and the direct influence of root and rhizomicrobial respiration on $CaCO_3$ recrystallization via control of CO_2 concentration and pH. Temperature, in contrast, was shown to exert indirect influence on this process: $CaCO_3$ recrystallization rates in planted loess strongly increased with ascending temperatures and were magnified by factor 10 between 10 °C and 30 °C in 'root-free' loess ($0.3 \cdot 10^{-5}$ day^{-1} vs. $3.1 \cdot 10^{-5}$ day^{-1}). This was attributed to enhanced water uptake by plants at high temperatures and subsequent stronger carbonate precipitation due to enhanced Ca^{2+} and HCO_3^- concentrations in soil solution, as well as higher root and rhizomicrobail respiration because of stronger growth. Periods necessary for complete recrystallization of loess $CaCO_3$ were calculated under the assumption of repeated recrystallization of primary and secondary $CaCO_3$ in 'root-free' loess prior to definite precipitation of secondary $CaCO_3$. Depending on length of growing season which in turn is controlled by climatic conditions, the time necessary for complete recrystallization of loess $CaCO_3$ was mainly in the range of 10^2 – 10^3 years. Closer relation of these findings to field conditions was ensured by taking into account the downward migration of recrystallized $CaCO_3$ by percolating water: 10^2 – 10^3 years were sufficient for carbonate leaching of upper soil horizons, but 10^3 – possibly 10^4 years were necessary for reprecipitation of secondary $CaCO_3$ in lower soil layers to form an accumulation horizon. In contrast, much faster

pedogenic carbonate formation (10^1 – 10^2 years) can occur in rhizosphere if calcification of roots leads to rhizolith formation, caused mainly by transpirational pull of the plant.

The recrystallization periods estimated by ^{14}C isotopic exchange represent minimum times, because the experimental design entailed rather high CO_2 concentrations in loess and thus overestimated recrystallization rates. Additionally, application of the here presented results to field conditions is restricted to soils derived from calcareous sedimentary parent material such as loess. In soils derived from solid rock or non-calcareous parent material, $CaCO_3$ recrystallization rates are limited by rates of rock weathering or of Ca^{2+} influx from external sources, respectively. Nevertheless, the results presented herein agree with literature data in terms of magnitude and demonstrate that the time frame of pedogenic carbonate formation comprises a broad spectrum from few decades up to tens of thousands of years. This has to be considered when using pedogenic carbonates as a tool to elucidate former environmental conditions based on stable isotope composition ($\delta^{13}C$, $\delta^{18}O$) or to assess the age of soils (^{14}C). The precision of such paleoenvironmental reconstructions is limited not by the analytical precision (IRMS for $\delta^{13}C$ or AMS for ^{14}C) but by the period necessary for secondary $CaCO_3$ formation and thus can be quite variable, depending on climatic conditions during pedogenic carbonate formation as well as on morphological appearance and position of the investigated $CaCO_3$ in the soil profile.

In this context, rhizoliths (calcified roots) are a powerful tool for paleoenvironmental studies, because they are probably formed within few decades, as indicated by modeled recrystallization periods. In the late Pleistocene loess-paleosol sequence of Nussloch, rhizoliths occur locally abundant with diameters up to 5 cm and length up to several m. They were not altered after formation, and their $\delta^{13}C_{carb}$ as well as $\delta^{13}C_{org}$ values indicate C_3 plants as source vegetation, which is also true for loess OM. However, the possible postsedimentary formation of rhizoliths was shown by the radiocarbon age of 3 kyears for one rhizolith sample, and enforced by different lipid composition of loess OM vs. rhizolith OM. The former derived from root and shoot biomass of grass and thus reflected former steppe conditions during loess deposition, whereas roots of shrubs or trees entered the loess later to form rhizoliths. Root exudates and associated microbial metabolites overprinted the composition of synsedimentary loess OM in the former rhizosphere on a molecular level, and potentially its original ^{13}C and ^{14}C isotope composition, by contributing considerable amounts of younger and qualitatively different OM. This circumstance can entail uncertainties for paleoenvironmental studies based on isotope composition of loess OM and likely applies also to other sediment-paleosol sequences.

Ongoing work focuses on the influence of further factors (e.g. soil moisture, compactness of primary $CaCO_3$), as well as on detailed assessment of the influence of root distance, on $CaCO_3$ recrystallization rates. Further, more rhizoliths and loess samples from the European Loess Belt will be investigated with respect to lipid and carbon isotope composition to elucidate source vegetation and to quantify postsedimentary input of root-derived OM in loess.

5 Contributions to the included manuscripts

This Ph.D. thesis comprises two publications and five manuscripts which were prepared in cooperation with various coauthors. The coauthors listed on these manuscripts contributed as follows:

Study 1: Effect of CO_2 concentration on the initial recrystallization rate of pedogenic carbonate – Revealed by ^{14}C and ^{13}C labeling

Status: Published in Geoderma, 2010, Vol. 155, page 351 – 358

M. Gocke: 70 % (experimental design, accomplishment of experiment, laboratory analyses, data preparation, manuscript preparation)

K. Pustovoytov: 10 % (discussion of results, comments to improve the manuscript)

Y. Kuzyakov: 20 % (discussion of experimental design and results, comments to improve the manuscript)

Study 2: Pedogenic carbonate recrystallization assessed by isotopic labeling: a comparison of ^{13}C and ^{14}C tracers

Status: Submitted to Journal of Plant Nutrition and Soil Science; date: 24.11.2009, revised version submitted 27.12.2010

M. Gocke: 70 % (experimental design, accomplishment of experiment, laboratory analyses, data preparation, manuscript preparation)

K. Pustovoytov: 8 % (discussion of results, comments to improve the manuscript)

Y. Kuzyakov: 22 % (discussion of results, comments to improve the manuscript)

Study 3: Carbonate recrystallization in root-free soil and rhizosphere of *Triticum aestivum* and *Lolium perenne* estimated by ^{14}C labeling

Status: Accepted for publication in Biogeochemistry; DOI: 10.1007/s10533-010-9456-z

M. Gocke: 70 % (experimental design, accomplishment of experiment, laboratory analyses, data preparation, manuscript preparation)

K. Pustovoytov: 10 % (discussion of results, comments to improve the manuscript)

Y. Kuzyakov: 20 % (discussion of experimental design and results, comments to improve the manuscript)

Study 4: Pedogenic carbonate recrystallization rates and periods depend on temperature-dependant rhizosphere processes: Relevance for paleoenvironmental applications

Status: Submitted to Geoderma; date: 20.12.2010

M. Gocke: 80 % (experimental design, laboratory analyses, data preparation, manuscript preparation)

Y. Kuzyakov: 20 % (discussion of experimental design and results, comments to improve the manuscript)

Study 5: Pedogenic carbonate formation: recrystallization vs. migration – process rates and periods assessed by ^{14}C labeling

Status: Submitted to Global Biogeochemical Cycles; date: 20.05.2010

M. Gocke: 63 % (experimental design, accomplishment of experiment, laboratory analyses, data preparation, manuscript preparation)

K. Pustovoytov: 10 % (discussion of results, comments to improve the manuscript)

Y. Kuzyakov: 27 % (discussion of experimental design and results, comments to improve the manuscript)

Study 6: Carbonate rhizoliths in loess and their implications for paleoenvironmental reconstruction – revealed by isotope composition: $\delta^{13}C$, ^{14}C

Status: Submitted to Chemical Geology; date: 07.03.2010, revised version submitted 08.12.2010

M. Gocke: 55 % (field work, laboratory analyses, data preparation, manuscript preparation)

K. Pustovoytov: 8 % (field work, discussion of results, comments to improve the manuscript)

P. Kühn: 7 % (micromorphological description of samples, discussion of results, comments to improve the manuscript)

G.L.B. Wiesenberg: 20 % (field work, data preparation, discussion of results, comments to improve the manuscript)

M. Löscher: 2 % (help in the field, comments to improve the manuscript)

Y. Kuzyakov: 8 % (discussion of results, comments to improve the manuscript)

Study 7: Rhizoliths in loess – evidence for postsedimentary incorporation of root-derived organic matter in terrestrial sediments assessed by molecular markers

Status: Published in Organic Geochemistry, 2010, Vol. 41, page 1198 – 1206

M. Gocke: 45 % (field work, laboratory analyses, data preparation, manuscript preparation)

Y. Kuzyakov: 10 % (discussion of results, comments to improve the manuscript

G.L.B. Wiesenberg: 45 % (field work, laboratory analyses, data preparation, manuscript preparation)

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II. Cumulative publications and manuscripts

Study 1: Effect of CO_2 concentration on the initial recrystallization rate of pedogenic carbonate – Revealed by ^{14}C and ^{13}C labeling

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Abstract

In calcareous parent material, pedogenic carbonate formation mostly involves dissolution and recrystallization of lithogenic carbonates with CO_2 of soil air, leading to a complete exchange of lithogenic carbon with soil-derived carbon. Interest in pedogenic carbonates has increased in recent decades because they are a useful tool for reconstructing paleoclimatic conditions ($\delta^{13}C$ and $\delta^{18}O$) and past atmospheric CO_2 concentrations as well as for radiocarbon dating of soils. For such investigations, the recrystallization rate of primary $CaCO_3$ by pedogenic carbonate formation and the dependence of the recrystallization rate on environmental factors are essential, but still unquantified factors.

The recrystallization rate of primary $CaCO_3$ of loess at three CO_2 concentrations was estimated by isotopic exchange between primary $CaCO_3$ and the ^{14}C of artificially labeled CO_2. Loess was used for the study as a parent substrate for soil formation to simulate initial rates of $CaCO_3$ recrystallization. CO_2 concentrations of 380 ppm, 5000 ppm and 50,000 ppm lead to recrystallization rates of $4.1 \cdot 10^{-7}$ day^{-1}, $8.1 \cdot 10^{-7}$ day^{-1} and $16.9 \cdot 10^{-7}$ day^{-1}, respectively. The relation between CO_2 concentrations and recrystallization rates was described by a saturation curve. Under the tested experimental conditions, complete (95%) recrystallization of loess carbonate and formation of pedogenic carbonate would take 4.9–$20.0 \cdot 10^3$ years, strongly depending on CO_2 concentration. We expect faster recrystallization rates under field conditions because of permanent CO_2 supply by root and rhizomicrobial respiration. This impedes the equilibrium between the inorganic C pools in solid, liquid and gaseous phases.

Keywords: pedogenic carbonate genesis; $CaCO_3$ recrystallization; CO_2; ^{14}C; loess; inorganic carbon

1 Introduction

Globally, soils contain a total of $659–748 \cdot 10^{15}$ g carbon (C) as $CaCO_3$ in the upper 1 m (Batjes, 1996). Sahrawat (2003) summarizes all carbonates in soils as the soil inorganic carbon (SIC) pool. In arid and semiarid regions, the SIC pool is the main C pool in terrestrial ecosystems, but under certain conditions (e.g. soil acidification) the pool might release CO_2 from soil and thus contribute to the greenhouse effect (Lal and Kimble, 2000).

Pedogenic carbonate is a typical product of soil formation under arid to semiarid climatic conditions. Over the last two decades, an increasing number of studies demonstrated the potential of pedogenic carbonate as a (paleo)environmental proxy, an indicator of past CO_2 concentrations and a chronological tool. The pedogenic carbonate is formed at carbon isotopic equilibrium between itself and soil CO_2 (Cerling, 1984; Nordt et al., 1996). Therefore, $\delta^{13}C$ reflects the photosynthetic pathway of the predominant local vegetation (Cerling et al., 1989; Amundson et al., 1989; Cerling and Quade, 1993). This fact served as the basis for reconstructions of paleovegetation (Quade and Cerling, 1995; Wang et al., 1996, 1997; Monger et al., 1998; Buck and Monger, 1999; Deutz et al., 2001; Pustovoytov et al., 2007). Moreover, diffusion models allow an estimation of former CO_2 concentrations in the Earth's

atmosphere (Cerling, 1991, 1992; Tanner et al., 2001). The $\delta^{18}O$ values of pedogenic carbonates are governed by the oxygen isotopic composition of meteoric water (Cerling, 1984), which inturn can reflect multiple factors (Cerling and Quade, 1993; Hsieh et al., 1998; Monger et al., 1998; Deutz et al., 2001). Finally, pedogenic carbonates are used as a tool for chronological studies (e.g. Vincent et al., 1994; Amundson et al., 1994; Wang et al., 1996; Pustovoytov, 2002; Pustovoytov et al., 2007).

One of the most important prerequisites for all these paleoenvironmental reconstructions and dating based on pedogenic carbonates is their complete recrystallization and preservation through time. Note, however, that pedogenic carbonate and other carbonate materials in soils can pass through diagenetic alteration, recrystallize and thus lose a substantial part of their initial stable isotopic and/or radiometric information (Cerling, 1991; Pendall et al., 1994; Nordt et al., 1998; Pustovoytov and Leisten, 2002; Budd et al., 2002).

Accurate paleoenvironmental reconstructions and chronological studies based on pedogenic carbonates require knowledge about the time scale of secondary carbonate formation. Up to now, however, the recrystallization rates of such carbonate, as well as the dependence of this rate on environmental factors (e.g. temperature and CO_2 concentration in soil air), remain unknown.

There are two basic approaches to assess the recrystallization rate of carbonate in soil. The first is to analyze the distribution of stable isotopic composition and/or radiocarbon age of carbonate over a soil profile of known age (Pendall et al., 1994; Pustovoytov and Leisten, 2002; Pustovoytov, 2003). The second approach is based on the rates of isotopic exchange under controlled conditions (Kuzyakov et al., 2006). It involves measuring the $^{14}CO_2$ that is photosynthetically assimilated by plants, respired by their root systems and associated microorganisms, and finally included by recrystallization into newly formed carbonate. The present study addresses certain unresolved questions in that latter work. Based on a linear increase of rhizosphere ^{14}C recovered in loess $CaCO_3$, Kuzyakov et al. (2006) calculated an initial recrystallization rate of $2.9 \cdot 10^{-5}$ day^{-1}. The authors concluded further that the time needed for complete recrystallization of $CaCO_3$ is at least 100, but probably 400–2000 years, depending on the assumptions when extrapolating the observed initial recrystallization rate. These estimations demonstrated the constraints for the chronological resolution of paleoenvironmental reconstructions based on $\delta^{13}C$ of pedogenic carbonate: evidently, the carbon isotopic signature of an earlier stage in the environmental history of a site can be replaced by a new isotopic signal relatively rapidly compared with the actual age of the proxy. The above study probably overestimated the recrystallization rate due to CO_2 accumulation within the plant pots, leading to a faster reaction between dissolved CO_2 and solid $CaCO_3$ than under field conditions.

$CaCO_3$ recrystallization is clearly affected by soil CO_2 concentration. Under field conditions, this concentration depends on vegetation and various soil properties, and varies between 0.035% and about 3.5% in volume (Davidson, 1995). The main aim of the present work was to determine the effects of CO_2 concentration as an isolated parameter, and the effects of the recrystallization period on the recrystallization rate of pedogenic carbonate

without plants. The method we apply is similar to that of Kuzyakov et al. (2006) and consists of quantifying the recrystallization rate of pedogenic carbonates by isotopic exchange with ^{14}C of artificially labeled soil CO_2.

^{14}C pulse labeling is a very sensitive approach, and thus suitable to estimate rates of very slow processes like $CaCO_3$ recrystallization. Using ^{14}C has the advantage of a very low detection limit (10^{-13} mol, by scintillation counting) compared to ^{13}C measurement (10^{-7} mol, by common mass spectrometry analyses). Nevertheless, in treatments with CO_2 concentrations higher than the atmospheric level, ^{13}C was applied simultaneously with ^{14}C to allow a comparison of the calculated recrystallization rates based on both ^{14}C and ^{13}C labeling.

2 Material and methods

2.1 Loess

Instead of soil, loess from a depth of 15 m below present surface was used for the experiment for the following reasons. Firstly, loess from this depth is not influenced by recent pedogenic processes. Therefore, the distribution of $CaCO_3$ is even and diffuse – no visual recrystallization took place – and the $CaCO_3$ crystals are as small as after initial loess formation. The loess contains little organic C; therefore, no significant microbial decomposition from organic sources can alter the CO_2 concentration in soil air. Secondly, contrary to most soils, loess has a high $CaCO_3$ content, which facilitates the recovery of applied ^{14}C. In summary, by using loess we simulated the initial steps of soil formation on typical loose silt loamy parent material containing $CaCO_3$. The used loesswas taken froman open cast mine at Nussloch (SW Germany, see Bente and Löscher, 1987) and contained 29% $CaCO_3$. For sedimentological and stratigraphic information on the loess from Nussloch, see Kuzyakov et al. (2006) and references therein.

2.2 Experiment layout

As experimental conditions, three time periods (4, 16 and 65 days) after the labeling and three CO_2 concentrations (380, 5000 and 50,000 ppm) were chosen.

For each replication, onemetal pipe (length 10.2 cm, inner diameter 1.6 cm) was filled with 29 g of air-dried and sieved loess. After moistening the loess to 70% of water holding capacity (WHC=28% of loess weight), each metal pipe was closed by connecting its ends with PVC tubings and a joint.

2.3 Labeling and sampling

For the labeling, 92.5 kBq of ^{14}C as $Na_2{}^{14}CO_3$ (ARC Inc., USA) was diluted with de-ionized water in a 30 ml vial. Previously, the water was slightly alkalinized to prevent loss of ^{14}C activity by exchange with atmospheric CO_2. Increasing amounts of 99% ^{13}C-enriched $Na_2{}^{13}CO_3$ (Isotec™, Ohio/USA) were added to the label solution, leading to the respective CO_2 concentrations above the atmospheric value of 380 ppm. The amounts of $Na_2{}^{13}CO_3$

necessary to achieve CO_2 concentrations of 5000 ppm (0.42 mg of $Na_2{}^{13}CO_3$ per sample) or 50,000 ppm (4.52 mg) were calculated by the air volume available within pore space of loess (0.32 ml per 1 g of loess minus the volume of added water) as well as within tubings, the membrane pump and the vial containing the label solution. After connecting themetal pipe to the label vial by PVC tubings, $^{14}CO_2$ and $^{13}CO_2$ were released by adding 3 ml of 5 M H_2SO_4 to the label solution and pumped through the loess sample for 10 min in a closed cycle by a membrane pump (Type SMG4, Gardner Denver Thomas GmbH, Germany) (Fig. 1-1a). After the labeling, the PVC tubings of the metal pipeswere closed by a joint. A small part of the labeled CO_2 stayed in the head space of the label vial and in the PVC tubings between that vial and the membrane pump. This unused $^{14}CO_2$ and $^{13}CO_2$ was trapped in 10ml of 1 M NaOH and considered for calculations based on ^{14}C activity.

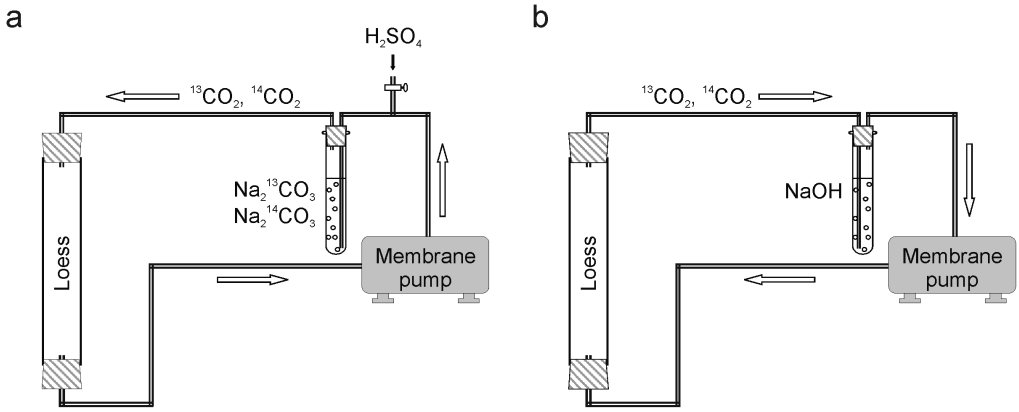

Fig. 1-1. Experimental setup. (a) Labeling procedure: ^{13}C and ^{14}C labeled CO_2 is released by addition of H_3PO_4 to the label solution and pumped through the loess sample. (b) trapping of gaseous CO_2 in NaOH before sampling of the loess.

The metal pipes then stayed closed for different time periods. As minimal experimental duration, we chose 4 days, because a previous study by Kuzyakov et al. (2006) had shown that $CaCO_3$ recrystallization is a rather slow process (rate $3 \cdot 10^{-5}$ day^{-1}) and we expected even lower values in our experiment without plants. A maximum time period of 65 days was chosen because this interval reflects the vegetative period in arid regions, i.e. the time in which CO_2 from root and rhizomicrobial respiration is involved in $CaCO_3$ recrystallization. The interval of 16 days was chosen as an intermediate period between those two. After 4, 16 or 65 days, the respective metal pipes were connected to a CO_2 trapping washing flask filled with 15 ml of 1 M NaOH and the air was pumped for 20 min (Fig. 1-1b). Thus, gaseous CO_2 remaining after recrystallization was removed from the loess sample and trapped in NaOH. Afterwards, the loess was pulled out from each metal pipe and carefully mixed. Five grams of loess were washed with 50 ml of slightly alkalinized de-ionized water to elute dissolved inorganic carbon (DIC), and dried at 90 °C for 24 h. Two grams of the dried loess were treated with 15 ml of 3 M H_3PO_4, and the CO_2 evolving from $CaCO_3$ was trapped in 12 ml of 2 M NaOH during 4 h to ensure complete CO_2 absorption. An aliquot of this NaOH was

titrated to assure that the total $CaCO_3$ content in loess had not changed during the experiment by formation of authigenic carbonate.

2.4 ^{14}C analysis

After the labeling, ^{14}C activities of the residue of the label solution and of the remaining CO_2 in NaOH were measured on 1 ml mixed with 2 ml of scintillation cocktail (Rotiszint EcoPlus, Carl Roth, Germany) after decay of chemiluminescence (for NaOH). The ^{14}C measurements were done by a 1450 LSC & Luminescence Counter (MicroBeta TriLux, Perkin Elmer Inc., USA). The ^{14}C counting efficiency was at least 70%; the measurement error did not exceed 3.5%. The absolute ^{14}C activity was standardized by SQP(I) by adding increasing amounts of NaOH as a quencher.

After opening themetal pipes, ^{14}C activity of CO_2 in NaOH and of DIC in water was measured on 1 ml aliquots as described above. The ^{14}C activity of loess carbonate, released as $^{14}CO_2$ by H_3PO_4 addition and trapped in NaOH, was measured on 6 ml aliquots added to 12 ml of scintillation cocktail. Larger aliquots were chosen for the ^{14}C analysis of samples with expected low ^{14}C activities (i.e. in loess carbonate). The ^{14}C counting efficiency of the used device (LS 6500 Multi-Purpose Scintillation Counter, Beckman, USA) was at least 90% and the measurement error did not exceed 4%. The absolute ^{14}C activity was standardized by the H number method, using a ^{137}Cs external standard.

2.5 $\delta^{13}C$ sample analysis

$\delta^{13}C$ analysis was conducted in loess samples in two analytical replications from each time period. $\delta^{13}C$ of loess carbonate was determined in amounts between 450 and 880 µg of loess on a Delta Plus XL isotope ratio mass spectrometer (Thermo Finnigan MAT, Bremen, Germany) connected to an elemental analyzer EA 3000 (Hekatech, Wegberg, Germany). $\delta^{13}C$ in carbonate of the initial loess as well as loess from the 380 ppm CO_2 treatment (no ^{13}C applied) were also measured. Results are given in ‰ relative to the V-PDB reference standard.

2.6 ^{14}C and ^{13}C calculation and statistical analysis

The ^{14}C results are presented as percentage of recovered ^{14}C activity. The total ^{14}C activity added to each replication ($^{14}C_{av}$) was calculated according to the equation:

$$^{14}C_{av} = {}^{14}C_{input} - {}^{14}C_{res} - {}^{14}C_{NaOH} \quad (1)$$

With $^{14}C_{input}$: total input activity of the label; $^{14}C_{res}$: activity of undissolved residue of label solution (percentage of input <1%); $^{14}C_{NaOH}$: activity of unused $^{14}CO_2$ trapped in NaOH.

The ^{14}C specific activity ($^{14}C_{SA}^{CO_2}$) of the label applied for each CO_2 concentration was calculated as the ratio of total input ^{14}C activity ($^{14}C_{input}$) and total C content in applied CO_2 (C_{total}^{input}):

$$^{14}C_{SA}^{CO_2} = \frac{^{14}C_{input}}{C_{total}^{input}} \quad (2)$$

The ^{14}C activity of the total amount of loess in each metal pipe ($^{14}C_{CaCO_3}$) was calculated from the ^{14}C activity of the 2 g of loess dissolved with H_3PO_4 (see previous chapter). The ^{14}C specific activity of the added CO_2 is equal to the ^{14}C specific activity of the recrystallized portion of $CaCO_3$. Therefore, the amount of recrystallized $CaCO_3$ ($C_{total}^{recryst}$) was calculated as:

$$C_{total}^{recryst} = \frac{^{14}C_{CaCO_3}}{^{14}C_{SA}^{CO_2}} \quad (3)$$

To calculate the recrystallization rate of the loess carbonate, the amount of incorporated C was divided by the amount of total C content of the loess carbonate ($C_{total}^{CaCO_3}$) and divided by the labeling period (4, 16, or 65 days) of the loess samples:

$$Rate = \frac{C_{total}^{recryst}}{C_{total}^{CaCO_3} \cdot t} \quad (4)$$

Recrystallization rates of the loess carbonate were also calculated by ^{13}C of CO_2 label accumulated within loess carbonate. For this purpose, $\delta^{13}C$ values from mass spectrometric analysis (‰) were converted into atomic % and the calculation was done based on ^{13}C mass balance.

The experiment was done with 4 replicates. Standard errors of means are presented in figures. Significance of differences between the treatments was analyzed by one-way ANOVA with α=5% significance level.

3 Results

3.1 ^{14}C distribution between the C pools

After addition of $^{14}CO_2$ to the loess and recrystallization, the maximal ^{14}C activity was recovered in loess $CaCO_3$ (except for the first sampling date at 50,000 ppm CO_2 concentration), followed by ^{14}C in DIC; the minimal ^{14}C activity was found in remaining CO_2 (Fig. 1-2). The part of ^{14}C recovered in DIC decreased from values between 47% and 55% after 4 days to values between 19% and 27% after 65 days. In contrast, an increasing amount of ^{14}C was recovered in loess carbonate. At the lowest CO_2 concentration (380 ppm), the part of ^{14}C recovered in loess carbonate increased from 52% after 4 days to 71% after 65 days. Values between the 4th and 65th day ascended from 49% to 80% at 5000 ppm and from 43% to 75% at 50,000 ppm CO_2 concentration. That means the ratio of $^{14}C_{CaCO_3}/^{14}C_{DIC}$ showed a stronger increase at enhanced CO_2 concentrations (5000 ppm, 50,000 ppm) compared to atmospheric CO_2 concentration (380 ppm).

3.2 $CaCO_3$ recrystallization rates and periods calculated based on ^{14}C incorporation

Within every single CO_2 concentration, the amount of recrystallized carbonate (as percent of total loess carbonate) did not change significantly over the 65-day experiment period. On the other hand, the applied CO_2 concentrations led to significantly different amounts of recrystallized carbonate. After 4 days, the amount of recrystallized C of loess carbonate was $1.6 \cdot 10^{-4}$%, $3.3 \cdot 10^{-4}$% and $6.8 \cdot 10^{-4}$% of initial $CaCO_3$-C for 380, 5000 and 50,000 ppm, respectively (Table 1-1). These values correspond to recrystallization rates of $4.1 \cdot 10^{-7}$ day^{-1}, $8.1 \cdot 10^{-7}$ day^{-1} and $16.9 \cdot 10^{-7}$ day^{-1} (Table 1-2).

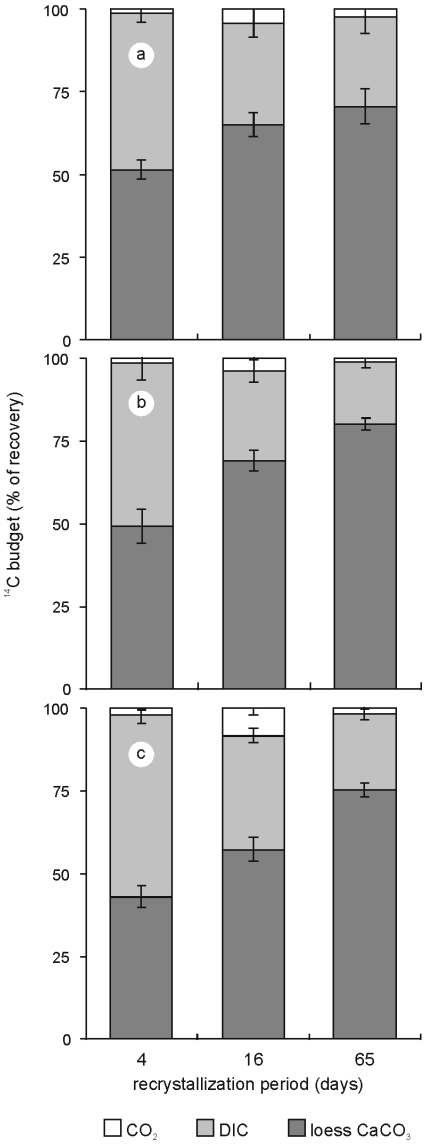

Fig. 1-2. ^{14}C distribution between calcium carbonate of loess, dissolved inorganic carbon, and gaseous CO_2 depending on the recrystallization period at three initial CO_2 concentrations of (a) 380, (b) 5000 and (c) 50,000 ppm.

Table 1-1

Amounts of recrystallized $CaCO_3$ (% of initial $CaCO_3$) after 4, 16 and 65 days under initial CO_2 concentrations of 380, 5000 and 50,000 ppm, calculated based on ^{14}C incorporation. SEM in brackets.

CO_2 (ppm)	380	5000	50000
4^{th} day	$1.6\ [\pm 0.1] \cdot 10^{-4}$	$3.3\ [\pm 0.7] \cdot 10^{-4}$	$6.8\ [\pm 0.5] \cdot 10^{-4}$
16^{th} day	$1.3\ [\pm 0.5] \cdot 10^{-4}$	$4.9\ [\pm 0.7] \cdot 10^{-4}$	$5.5\ [\pm 0.7] \cdot 10^{-4}$
65^{th} day	$1.0\ [\pm 0.1] \cdot 10^{-4}$	$2.4\ [\pm 0.1] \cdot 10^{-4}$	$7.2\ [\pm 0.2] \cdot 10^{-4}$

Table 1-2

Recrystallization rates (day^{-1}) calculated based on ^{13}C and ^{14}C incorporation. SEM in brackets. For ^{13}C, the recrystallization rate could not be calculated at 380 ppm CO_2 concentration (or at 5000 ppm CO_2 concentration after a recrystallization period of more than 4 days) because the $\delta^{13}C$ values of the respective replications were lower than the value of unlabeled loess.

	1^{st} sampling date (4^{th} day)		**2^{nd} sampling date (16^{th} day)**	
CO_2 (ppm)	^{13}C	^{14}C	^{13}C	^{14}C
380	nd	$4.1\ [\pm 0.1] \cdot 10^{-7}$	nd	$0.8\ [\pm 0.3] \cdot 10^{-7}$
5000	$3.2\ [\pm 1.0] \cdot 10^{-6}$	$8.2\ [\pm 1.7] \cdot 10^{-7}$	nd	$3.1\ [\pm 0.4] \cdot 10^{-7}$
50000	$13.8\ [\pm 6.0] \cdot 10^{-6}$	$16.9\ [\pm 1.0] \cdot 10^{-7}$	$6.2\ [\pm 3.9] \cdot 10^{-6}$	$3.4\ [\pm 0.4] \cdot 10^{-7}$

As the amount of recrystallized carbonate did not change significantly over time within each CO_2 treatment, the average amount in every single

CO_2 treatment over the whole recrystallization period is presented in Fig. 1-3 against CO_2 concentration. The values increased with increasing CO_2 concentrations; the curve showed a rather steep rise under low CO_2 concentrations (up to a few thousand ppm), but was less steep at very high concentrations. We used a simple equation to calculate the dependence of the amount of recrystallized $CaCO_3$ on the CO_2 concentrations and fitted the parameters by non-linear regression (Eq. (5)). As a recrystallization period < 4 days was unimportant for the amount of $CaCO_3$ recrystallized,we did not include time in the equation. Based on this equation with fitted parameters we roughly estimated the recrystallized $CaCO_3$ amounts ($C_{total}^{recryst}$) depending on CO_2 concentration (CO_2) in soil air as:

$$C_{total}^{recryst} = 0.000544 \cdot (1 - e^{-0.000117 \cdot CO_2}) + 0.00011 \quad (5)$$

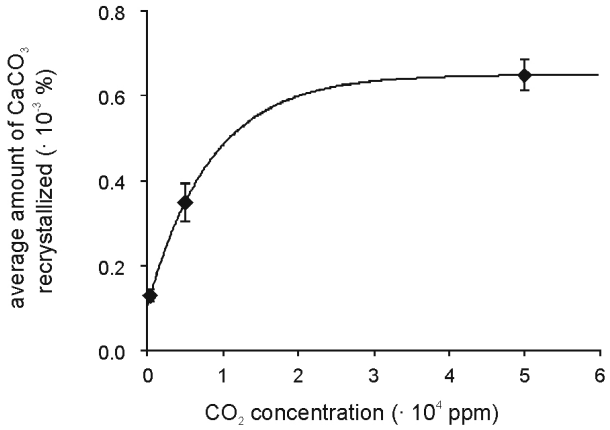

Fig. 1-3. Amount of recrystallized calcium carbonate as percentage of total loess carbonate (averaged from all three sampling dates) depending on CO_2 concentration.

From these initial rates, the time necessary for complete $CaCO_3$ recrystallization can be estimated in two ways. The first assumes that the once-formed secondary carbonate is not affected by recrystallization again, leading to a linearly increasing amount of recrystallized carbonate. With this approach, the $CaCO_3$ of loess with 29% carbonate content will be completely recrystallized after approximately 6300, 3200 or 1500 years at a CO_2 concentration of 380, 5000 or 50,000 ppm, respectively (Table 1-3).

Table 1-3

Calculated periods (rounded up to ten years) necessary for the recrystallization of 95% of initial loess carbonate (for loess containing 29% $CaCO_3$). 95% Confidence intervals of the recrystallization periods are given in brackets.

	380 ppm	5000 ppm	50000 ppm
linear[a]	5990 years [5630 – 6390]	3190 years [2260 – 5400]	1540 years [1350 – 1780]
exponential[a]	18880 years [17760 – 20150]	10050 years [7130 – 17030]	4840 years [4270 – 5600]

[a] Linear or exponential decrease in the amount of remaining primary $CaCO_3$. These two approaches are represented by straight and curved lines in Fig. 1-5.

The second approach assumes that both the primary loess carbonate and the secondary carbonate will react repeatedly with soil air CO_2. Thus, the amount of remaining, not recrystallized carbonate ($CaCO_3(t)$) exponentially decreases, depending on time and recrystallization rate (Eq. (6)). In this case, CO_2 concentrations of 380, 5000 or 50,000 ppm will lead to complete $CaCO_3$ recrystallization periods of 20,000, 10,000 or 4900 years, respectively (Table 1-3).

$$CaCO_3(t) = 100 \cdot e^{-t \cdot rate} \quad (6)$$

3.3 $\delta^{13}C$ values of loess carbonate and resulting recrystallization rates

Calcium carbonate of the original, unlabeled loess from Nussloch showed a ^{13}C natural abundance of −1.59±0.40‰. At both lowest CO_2 concentrations, no significant change of $\delta^{13}C$ values of $CaCO_3$ occurred over time: samples of the 380 ppm CO_2 treatment (no ^{13}C applied) plotted very near to the initial value, with−1.71‰ after 4 days and −1.67‰ after 16 days. At a CO_2 concentration of 5000 ppm, $\delta^{13}C$ values plotted only slightly above the value of unlabeled loess, with −0.41‰ after 4 days and −1.16‰ after 65 days (Fig. 1-4). After 16 days the value was lower (−2.09‰). These $\delta^{13}C$ changes over time were not significant. At the highest applied CO_2 concentration (50,000 ppm), $\delta^{13}C$ increased after 4 days up to +3.44‰ and at 16th day up to +7.41‰, then decreased again to +0.12‰.

The recrystallization rate of carbonate was calculated based on the accumulation of ^{13}C for samples of the 5000 ppm treatment (first sampling date) and 50,000 ppm treatment (first and second sampling date) because only these samples showed $\delta^{13}C$ values considerably above the value of unlabeled loess. The last sampling date (65th day) was not included because of the strong decrease of $\delta^{13}C$ values between day 16 and the end of the experiment.

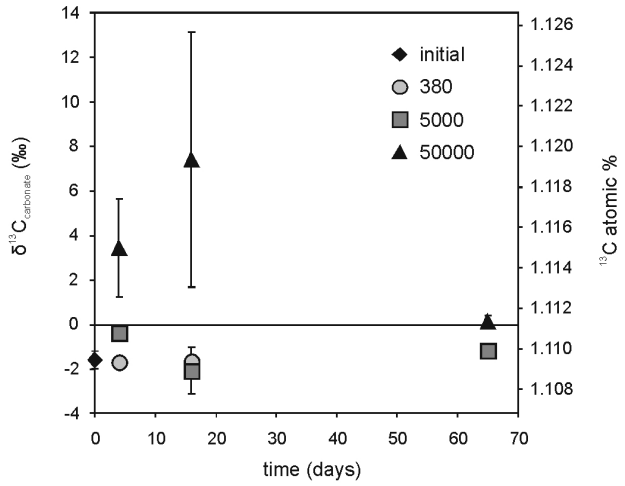

Fig. 1-4. Change of $\delta^{13}C$ values depending on time and CO_2 concentration. The secondary axis represents the ^{13}C atomic % calculated from $\delta^{13}C$ values.

In general, the recrystallization rates calculated based on ^{13}C accumulation were one order of magnitude higher than the results of the ^{14}C calculation (Table 1-2). Recrystallization rates

based on ^{13}C and ^{14}C from the 5000ppm treatment could be compared only for the first sampling date, because the $\delta^{13}C$ value after 16 days plotted below that of unlabeled loess (Fig. 1-4).

4 Discussion

4.1 Isotopic exchange approach

The isotopic exchange between C of loess carbonate and artificial CO_2 label was used to estimate the recrystallization rate of loess $CaCO_3$. Both applied C isotopes, ^{13}C as well as ^{14}C, showed that labeled C was incorporated into the loess carbonate by recrystallization. However, calculation with either ^{14}C activity or ^{13}C enrichment within recrystallized loess carbonate yielded different results. As the calculated amounts of recrystallized carbonate were very small (0.00008–0.00076% of total loess $CaCO_3$), a very sensitive method is necessary for short recrystallization periods such as in our experiment. The measurement accuracy of a mass spectrometric analysis is between 0.2‰ and 0.5‰. A variation of a few ‰ between replications of the same treatment can occur due to inhomogeneous distribution of ^{13}C incorporated into $CaCO_3$. Such a variation of a few ‰ leads to differences in the estimated recrystallization rate of up to one order of magnitude. Results calculated based on ^{14}C activity showed that the applied CO_2 concentrations led to differences of recrystallization rates between the CO_2 treatments of one order of magnitude or less. Thus, the variation of the ^{13}C approach equals or even exceeds the differences between treatments estimated by the ^{14}C approach: the latter is therefore more accurate. The two lower CO_2 concentrations (380 and 5000 ppm) did not lead to significant changes in $\delta^{13}C$, although artificial ^{13}C (99%) was applied only to the latter one (Table 1-2). We assume that C of added $^{13}CO_2$ was incorporated into loess carbonate by recrystallization, similarly to the ^{14}C approach, also in the 380 ppm treatment. However, these changes in $\delta^{13}C$ are presumably too small to be detected by mass spectrometry. We therefore conclude that the sensitivity of ^{13}C measurements was neither high enough to estimate such slow recrystallization rates, nor to reveal small differences between the treatments. Accordingly, the isotopic exchange based on ^{14}C is probably the only possibility to estimate such slow processes rates. The further discussion therefore focuses only on ^{14}C labeling results.

The reason for the $\delta^{13}C$ decrease towards the last sampling date remains unknown. We cannot rule out that microbial processes in the loess influenced the $\delta^{13}C$ value after 65 days.

4.2 ^{14}C distribution and equilibria between C pools

According to the Henry Law, the solubility of CO_2 in water increases directly proportional to the CO_2 partial pressure in soil air. As dissolution of soil $CaCO_3$ depends on the pH of the soil solution and thus on CO_2 partial pressure, increasing CO_2 concentrations should result in rising amounts of recrystallized $CaCO_3$. Depending on the depth in the soil profile and biological activities by plants and microorganisms, the CO_2 concentration in soil air ranges between atmospheric values (approximately 0.038% in volume) and values one or two orders of magnitude larger than in the atmosphere (typically up to 3.5% in volume, Davidson, 1995).

To test the effect of CO_2 concentration on the recrystallization rate of pedogenic carbonates, we applied three treatments covering approximately the natural range of CO_2 concentrations in soil air.

The CO_2 concentration strongly influenced the carbonate recrystallization rate (Eq. (5), Fig. 1-3). The three CO_2 concentrations in pore space led to three distinct distribution patterns of ^{14}C from the input CO_2 between solid $CaCO_3$, dissolved inorganic carbon (DIC) and gaseous CO_2 within the loess samples. Given a constant CO_2 concentration, the reaction between gaseous and liquid phase (Eq. (7)) rapidly reaches an equilibrium (seconds to minutes). For this reason and due to the immediate contact between labeled CO_2 and loess pore water, the least recovered ^{14}C was found in the gaseous CO_2 compartment, and it stayed in the same percentage range of total recovered ^{14}C during all labeling periods. In contrast, more time is required to establish an equilibriumfor the reaction between liquid and solid phase (Eq. (8), leading to C exchange in dissolved form). This time span could not be assessed in our study, but it should be less than 4 days (probably several hours to a few days) because the amount of recrystallized $CaCO_3$ did not change significantly after 4, 16 or 65 days. Therefore, the calculated rates have to be seen as minimum values, which however point out the order of magnitude of carbonate recrystallization rates under the conditions given in our experiment.

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \quad (7)$$

$$H_2CO_3 + CaCO_3 \leftrightarrow Ca^{2+} + 2HCO_3^- \quad (8)$$

4.3 Recrystallization periods of loess carbonate

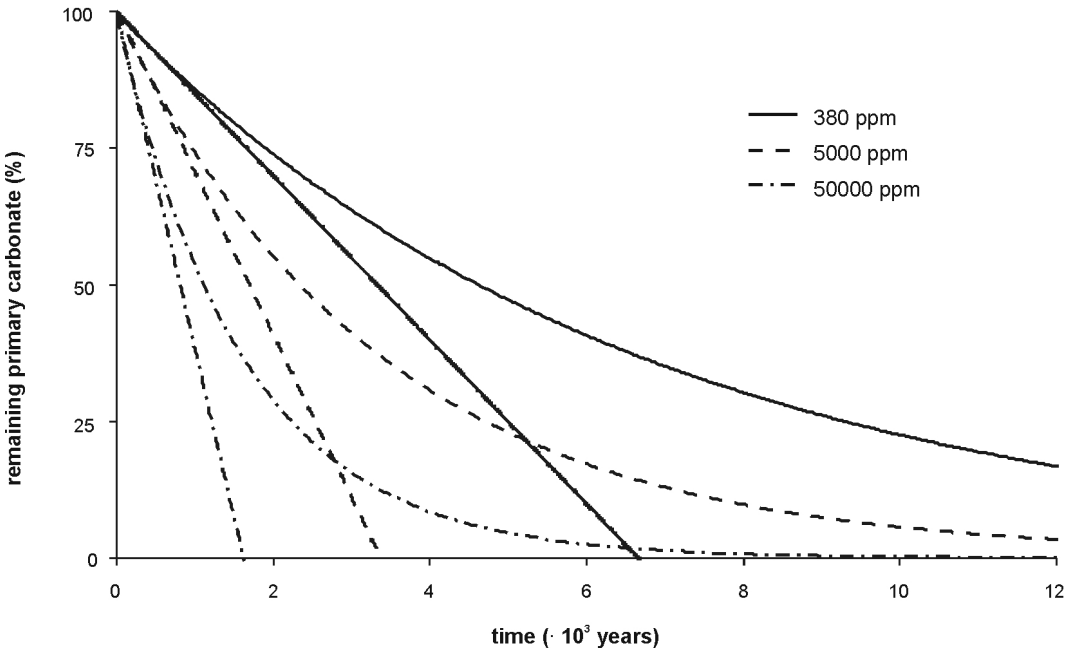

Fig. 1-5. Decrease in the amount of remaining primary carbonate over time, calculated separately for the three CO_2 concentrations. The straight lines were calculated by presuming that loess carbonate was recrystallized once. In contrast, the exponential lines represent the condition that $CaCO_3$ is affected several times by the recrystallization process (see text).

A linear and an exponential approach were used to calculate the decrease in the amount of remaining primary carbonate. The linear approach led to modeled recrystallization periods of 1500–6300 years for the CO_2 concentrations applied in this study (Fig. 1-5, straight lines). Such a linear recrystallization process is possible only in the presence of progressively growing $CaCO_3$ crystals. Without an irreversible $CaCO_3$ crystal growth, the fine-spread carbonate will be repeatedly recrystallized with pore-space CO_2, causing the decrease in the amount of not recrystallized carbonate to exponentially decelerate over time (Fig. 1-5, exponential lines). This is the most likely mode of carbonate recrystallization in real soil systems. In the case of the exponential approach, full (95%) carbonate recrystallization of the exposed loess carbonate takes approximately 20,000, 10,000 and 4900 years at 380, 5000 and 50,000 ppm CO_2 concentration, respectively (Table 1-3).

4.4 Relevance of the estimated recrystallization rates

The ^{14}C labeling method was first applied to estimate pedogenic carbonate recrystallization rates by Kuzyakov et al. (2006). The authors exposed wheat to an artificially labeled ^{14}C atmosphere to estimate the amount of root-derived C incorporated into loess carbonate. The initial recrystallization rate of $2.9 \cdot 10^{-5}$ day^{-1} they calculated is 1–2 magnitudes of order higher than the results of the present study (calculated based on ^{14}C isotopic exchange), although we applied the relevant CO_2 concentrations. In the experiment with plants by Kuzyakov et al. (2006), various factors may have contributed to the faster recrystallization. Permanent CO_2 production by root and rhizomicrobial respiration hindered a steady state between CO_2 and CO_3^{2-} in the liquid and solid phase (Eqs. (7) and (8)). The continuous flux of CO_2 into soil thus promotes carbonate recrystallization. Additionally, plants change the chemical environment in the soil: a decreased pH, resulting from root exudates, led to a faster C isotope exchange compared to the present study without plants and with one-time CO_2 supply.

Furthermore, soil CO_2 profiles and CO_2 fluxes are in a complex relationship with environmental factors like CO_2 production, soil water content, soil temperature and gas diffusivity (Hashimoto and Komatsu, 2006). In upper soil horizons, CO_2 production is controlled mainly by vegetation. Spatial CO_2 distribution in a soil depends on morphological features of subsurface plant biomass, such as root thickness and root distribution within the soil profile (Hamada and Tanaka, 2001). As the CO_2 concentration decreases with increasing distance to the root surfaces, our results suggest highest carbonate recrystallization rates in the rhizosphere (the soil volume directly affected by processes of living plants; definition by Darrah, 1993). Temporal differences in CO_2 concentration, on the other hand, occur due to specific growing seasons per year, dependent on plant species andenvironmental factors like light intensity, temperature and moisture (e.g. Russo and Knapp, 1976). Carbonate recrystallization in soils is expected to be higher during the plant growing season than in winter months. Moreover, CO_2 concentration in soil is controlled by the plants' growth rate, which differs considerably between grassland vegetation, agricultural crops and trees, and by the portion of assimilates used by the plant for rhizosphere processes (reviewed by Whipps, 1990; Kuzyakov and Domanski, 2000). As an example, ryegrass (*Lolium perenne*), a typical

representative of grasslands under humid and semiarid climate, grows very slowly and puts a major part of the assimilates into rhizosphere respiration (Meharg and Killham, 1990, 1991). Maize (*Zea maize*), on the other hand, invests most of the photoassimilates for above-ground production (Todorovic et al., 2001; Kuzyakov and Cheng, 2004). The higher growth rate of maize might result in a higher recrystallization rate compared to grassland, but in the long-term, such differences could be compensated by stronger rhizosphere respiration and longer vegetation periods of pasture plants.

Temperature is another important factor governing the $CaCO_3$ equilibrium. On one hand, higher temperature leads to decreasing CO_2 solubility, thus diminished carbonate recrystallization should be expected. However, the role of temperature for several biological processes ismuch higher than its influence on the chemical equilibrium between CO_2, HCO_3^- and $CaCO_3$. Root respiration, exudation and microbial respiration are promoted by increasing temperature, boosting CO_2 production and $CaCO_3$ recrystallization. Moreover, higher temperature enhances plant and soil evapotranspiration and leaching (Lal and Kimble, 2000). The resulting variations in soil moisture might also contribute to faster carbonate recrystallization.

Soil texture (particle size distribution, clay content), aggregates and pore space geometry play an important role for gas diffusivity. According toKawamoto et al. (2006), soil permeability generally increases from finer (sandy clay loam) to coarser (sand) textured soil but in many cases tends to be lowest in sandy loam soils. Another aspect of soil texture is the adsorption of Ca^{2+} by clay minerals (Scharpenseel et al., 2000), thus removing the reactant necessary for re-precipitation of pedogenic $CaCO_3$. Accordingly, soil with high clay content could restrict carbonate recrystallization directly and indirectly.

This study gives a first insight into the direct effect of soil CO_2 concentration on carbonate recrystallization rates in potential soil environment without influence of rhizosphere. To provide permanent control on the state of isotopic composition of carbonate, we operated with loess samples in closed tubes, which is not a direct analogue to a soil milieu. However, our experiments involved components available in real soil systems: loess particles as solid phase, carbon dioxide as gas phase and water as fluid phase. Two aspects of the study are important to be seen. First, the interaction between CO_2 and soil is not complicated by specific effects of root systems. It helps to better understand the abiotic component of carbonate recrystallization in soils. Second, the influence of CO_2 on carbonate in the absence of plant roots theoretically can take place in some rare soil environments such as soils of extreme deserts, toxically affected soils, extraterrestrial soils etc.

We showed that the rate increases with increasing CO_2 concentration. This supports the assumption that firstly, the rate should be higher in planted than in unplanted soil, and secondly, higher in root vicinity than in soil distinct to roots due to CO_2 release by root and rhizomicrobial respiration. In future studies we will expose plants to an artificially labeled atmosphere (as previously demonstrated by Kuzyakov et al., 2006) to show the incorporation of root-derived C into newly formed secondary carbonate under different environmental conditions.

5 Conclusions

As a very sensitive method, ^{14}C labeling is a useful tool to assess slow rates of steady state processes in soils; it is probably the only approach for estimating the recrystallization rate of pedogenic $CaCO_3$.

Rising CO_2 concentration increases the CO_2 partial pressure, enhancing the dissolution and recrystallization of calcium carbonate from loess. Therefore, under field conditions the CO_2 concentration in soil air, ranging fromatmospheric values up to approximately 100 times the atmospheric level, affects $CaCO_3$ recrystallization rates remarkably. The relation between the amount of recrystallized $CaCO_3$ and the CO_2 concentration is described by a saturation curve. In our study, CO_2 concentrations of 380, 5000 and 50,000 ppm led to initial recrystallization rates of $4.1 \cdot 10^{-7}$ day^{-1}, $8.1 \cdot 10^{-7}$ day^{-1} and $16.9 \cdot 10^{-7}$ day^{-1}. Assuming an exponential decrease of the remaining primary loess carbonate due to repeated reaction of the secondary carbonates with CO_2, full (95%) recrystallization of the loess carbonatewould take 4900–20,000 years. In soil under growing plants, however, much higher recrystallization rates (at least 1–2 orders of magnitude) and thus shorter recrystallization periods occur due to permanent CO_2 production by root and rhizomicrobial respiration.

Further research is necessary to elucidate the effect of biotic and abiotic factors like depth below the soil surface, properties of carbonate material, plant species, moisture, temperature or carbonate content of soil on the recrystallization rate of pedogenic carbonates.

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Study 2: Pedogenic carbonate recrystallization assessed by isotopic labeling: a comparison of ^{13}C and ^{14}C tracers

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Abstract

The carbon isotopic composition ($\delta^{13}C$) of pedogenic carbonates reflects the photosynthetic pathway of the predominant local vegetation because pedogenic (secondary) $CaCO_3$ is formed in isotopic equilibrium with soil CO_2 released by root and rhizomicrobial respiration. Numerous studies show the importance of pedogenic carbonates as a tool for reconstructing paleoecological conditions in arid and semiarid regions. The methodological resolution of these studies strongly depends on the time scale of pedogenic carbonate formation, which remains unknown. The initial formation rate can be assessed by ^{14}C labeling of plants grown on loess and subsequent incorporation of ^{14}C from rhizosphere CO_2 into newly formed carbonate by recrystallization of loess $CaCO_3$. We tested the feasibility of ^{14}C and ^{13}C tracers for estimating $CaCO_3$ recrystallization rates by simultaneous ^{14}C and ^{13}C labeling and comparison with literature data. ^{14}C labeling was more efficient and precise in assessing recrystallization rates than ^{13}C labeling. This is connected with higher sensitivity of ^{14}C liquid scintillation counting when compared with $\delta^{13}C$ measurement by IRMS. Further, assessment of very low amounts of incorporated tracer is more precise with low background signal (natural abundance), which is true for ^{14}C, but is rather high for ^{13}C. Together, we obtained better reproducibility, higher methodological precision and better plausibility of recrystallization rates calculated based on ^{14}C labeling. Periods for complete $CaCO_3$ recrystallization, extrapolated from rates based on ^{14}C labeling, ranged from 130 (125 – 140) to 240 (225 – 255) years, while it was about 600 (365 – 1600) years based on the ^{13}C approach. In terms of magnitude, data from late Holocene soil profiles of known age provide better fit with modeled recrystallization periods based on the ^{14}C approach.

Key words: secondary carbonate / $CaCO_3$ recrystallization / soil inorganic carbon / isotopic pulse labeling / rhizosphere / loess

1 Introduction

Soils of arid and semiarid regions show favorable conditions for precipitation of secondary carbonates (*Borchardt* and *Lienkaemper*, 1999). These carbonates serve as an important tool for paleoenvironmental and/or paleoclimatic reconstructions (e.g. *Quade* and *Cerling*, 1995, *Buck* and *Monger*, 1999, *Mora* and *Pratt*, 2001, *Kaakinen* et al., 2006, *Pustovoytov* et al., 2007a). Pedogenic carbonates can also be used for dating soils and paleosols based e.g. on their radiocarbon age (*Amundson* et al., 1994, *Pustovoytov* et al., 2007b) or thickness of secondary carbonate coatings on pebbles (*Pustovoytov*, 2003, *Amoroso*, 2006). Furthermore, they provide insights into former atmospheric CO_2 concentrations (e.g. *Tanner* et al., 2001, *Royer*, 2006). The prerequisite for conclusions based on these studies is that secondary carbonates form in isotopic equilibrium with CO_2 from soil air (*Cerling*, 1984, *Cerling* et al., 1989), released mainly by root and rhizomicrobial respiration (*Amundson* et al., 1998). Therefore, the C isotope composition of pedogenic carbonates comprises information about the vegetation present during their formation (*Nordt* et al., 1996). When regarding sedimentary environments, most authors agree that precipitation

of pedogenic carbonates does not involve significant amounts of CO_3^{2-} from primary material (e.g. *Cerling*, 1984, *Quade* et al., 1989). However, the prerequisite for this process is the presence of Ca^{2+} in the soil solution, derived either from external (dust, rainfall) or internal sources (weathering of Ca bearing minerals in parent material; *Birkeland* 1999). In case of calcareous soil parent material like e.g. loess, Ca^{2+} is provided solely from dissolution of primary loess $CaCO_3$, because in the presence of $CaCO_3$, weathering of other soil minerals is impossible and consequently, there is no other source for Ca^{2+}. This means that loess $CaCO_3$ is dissolved and, after C isotopic exchange with soil air CO_2 and subsequent drying of soil, reprecipitated as pedogenic $CaCO_3$.

Despite increasing scientific interest in pedogenic carbonates, long-term $CaCO_3$ recrystallization processes in soils and paleosols remain poorly understood. However, knowledge of the long-term dynamics of secondary carbonate (10^4–10^8 years) would be essential for the precision of geochronological and paleoenvironmental studies based on pedogenic $CaCO_3$ (*Cerling*, 1991, *Amundson* et al., 1994, *Royer* et al., 2001). Previous attempts to assess this problem are based on abundances of C isotopes in naturally formed secondary carbonates: $\delta^{13}C$ and $\Delta^{14}C$ (*Pendall* et al., 1994) and in dated artificial carbonate material (*Pustovoytov* and *Leisten*, 2002). Analysis of ^{13}C natural abundance in pedogenic carbonates is not sensitive enough to reveal small changes in isotopic signatures resulting from isotopic exchange. Moreover, studies based on radiocarbon ages can only roughly estimate the time frame of isotopic re-equilibration between carbonates and respired CO_2 in the uppermost soil horizons. Our understanding of this process is complicated by the very long periods necessary for secondary carbonate formation. Altogether, no one has yet determined the initial rate of secondary carbonate formation in situ.

A new approach for estimating the initial recrystallization rate of pedogenic carbonates under controlled conditions was proposed by *Kuzyakov* et al. (2006): repeated ^{14}C pulse labeling of plants grown on loess. Based on the isotopic exchange between primary loess $CaCO_3$-C and C from respired CO_2, the ^{14}C assimilated by plants, respired by roots and rhizomicrobial organisms and incorporated in secondary $CaCO_3$ was quantified in the loess $CaCO_3$. This estimate of the amount of root-derived C incorporated into loess carbonate by recrystallization yielded an initial recrystallization rate of $3 \cdot 10^{-5}$ day^{-1} as part of the total loess carbonate. By extrapolation, the authors concluded that several hundreds to a few thousands of years were necessary for complete recrystallization of the primary loess carbonate in the uppermost soil horizons.

In recent decades, ^{14}C and/or ^{13}C pulse labeling of plants has been applied to a variety of soil- and plant-related topics, e.g. tracing of C allocation by plants into soil (reviewed by *Kuzyakov*, 2001), whereas ^{14}C was preferred in most studies because of its high sensitivity, lower costs of purchase and analyses and more convenient sample preparation (*Kuzyakov* and *Domanski*, 2000). In the case of pedogenic carbonate formation, only ^{14}C labeling of plants has been applied to estimate the recrystallization rate of pedogenic carbonates (*Kuzyakov* et al., 2006), an approach that turned out to be highly reproducible (*Gocke* et al., 2010a). Another study dealing with the initial recrystallization rate of pedogenic carbonates compared

the reliability of ^{13}C and ^{14}C labeling without plants, but by direct contact between primary carbonate (from loess) and dual-labeled (^{13}C, ^{14}C) CO_2 in closed system (*Gocke* et al., 2010b). The results argued for the preference of ^{14}C over ^{13}C for studies, because the data calculated based on ^{14}C were more consistent. Recrystallization rates obtained by ^{14}C labeling without plants were one to two orders of magnitude lower (10^{-6}; *Gocke* et al., 2010b) than with plants (10^{-5} – 10^{-4}; *Kuzyakov* et al., 2006). This is most probably due to the permanent CO_2 supply in planted loess by root and rhizomicrobial respiration. Therefore, we expected that higher recrystallization rates of $CaCO_3$ in the presence of plants will allow also application of ^{13}C labeling for the estimation of periods of pedogenic carbonate formation, which was not tested so far.

This study compares the potential of two C tracers for the isotopic exchange approach – ^{13}C and ^{14}C – to assess the initial rates of initial carbonate recrystallization by pulse labeling. For this purpose, we labeled plants in atmosphere with $^{13}CO_2$ and $^{14}CO_2$ and compared the carbonate recrystallization rates obtained based on both tracers.

2 Material and methods

2.1 Experimental layout and labeling

Plants were grown in vessels with three inlets in the lid and one main opening for growth of the plant shoots (CombiSart, Sartorius AG, Fig. 2-1a). Each vessel was filled with 450 g of air-dried and sieved loess ($CaCO_3$ content 29.0%) from Nussloch, SW Germany. Loess was chosen because of uniform distribution of fine carbonate and very low content of organic material, thereby simulating initial conditions of pedogenesis on a sedimentary calcareous material. Moreover, the high primary $CaCO_3$ content of loess leads to carbonate recrystallization without formation of additional $CaCO_3$, because primary loess calcite represents the major Ca^{2+} source for secondary $CaCO_3$, while further Ca^{2+} bearing minerals like feldspar or some mafic minerals (e.g. mica, amphiboles) cannot be weathered in the presence of $CaCO_3$.

Three vessels were planted with wheat [*Triticum aestivum* (L.)] and three with ryegrass [*Lolium perenne* (L.)]. For nutrient supply, modified Hoagland nutrient solution (Hoagland and Arnon, 1950) was added, and loess moisture was set to 70% of water holding capacity (100% WHC = 28% of loess weight). After a growth period of 27 days for wheat and 59 days for ryegrass, the vessels were flushed with air to remove CO_2 accumulated in the vessels by root and rhizomicrobial respiration prior to the labeling. The openings of the plant pots were then completely sealed to prevent loss of labeled and total CO_2 released by root and rhizomicrobial respiration. The aboveground plant parts were pulse-labeled simultaneously in $^{14}CO_2$ and $^{13}CO_2$ atmosphere, with the ^{13}C isotopic label consisting of 10 mg of 99% enriched $Na_2{}^{13}CO_3$ per plant pot with wheat and 5 mg for ryegrass (resulting in ^{13}C enrichment in the CO_2 of the atmosphere of about 44% and 28% ^{13}C, respectively). The ^{14}C activity was 407 kBq per plant pot. Plant growing conditions and the labeling technique were described in

detail by *Kuzyakov* et al. (2006) and *Gocke* et al. (2010a). Before and after the pulse labeling, the plants were grown under normal atmospheric conditions.

2.2 Analyses

Between the labeling and the sampling, CO_2 released by root and rhizomicrobial respiration was not flushed out. This allowed CO_2 accumulation in the loess-root compartment and the isotopic exchange between respired CO_2 and loess $CaCO_3$ by recrystallization. Five days after the labeling, CO_2 from root and rhizomicrobial respiration was pumped out and trapped in 15 ml of 1 M NaOH (Fig. 2-1b). This time interval between labeling and sampling was chosen, because it is long enough to allow for release of the major part of previsouly assimilated C tracer by roots (≤ 3 days; *Kuzyakov* and *Cheng*, 2004) as well as for isotopic exchange between primary $CaCO_3$ and respired CO_2 (≤ 4 days; *Gocke* et al., 2010b), and short enough to avoid O_2 limitation in the loess-root compartment. At the sampling date, the plants were cut at the base, and the content of the CombiSart device was divided into roots and loess (non-rhizosphere loess) by tweezers. The roots were washed, and loess remaining in the washing water, originating from the proximity of the roots or root surface (in the following termed rhizosphere loess), was filtrated and dried at 90 °C for 24 h.

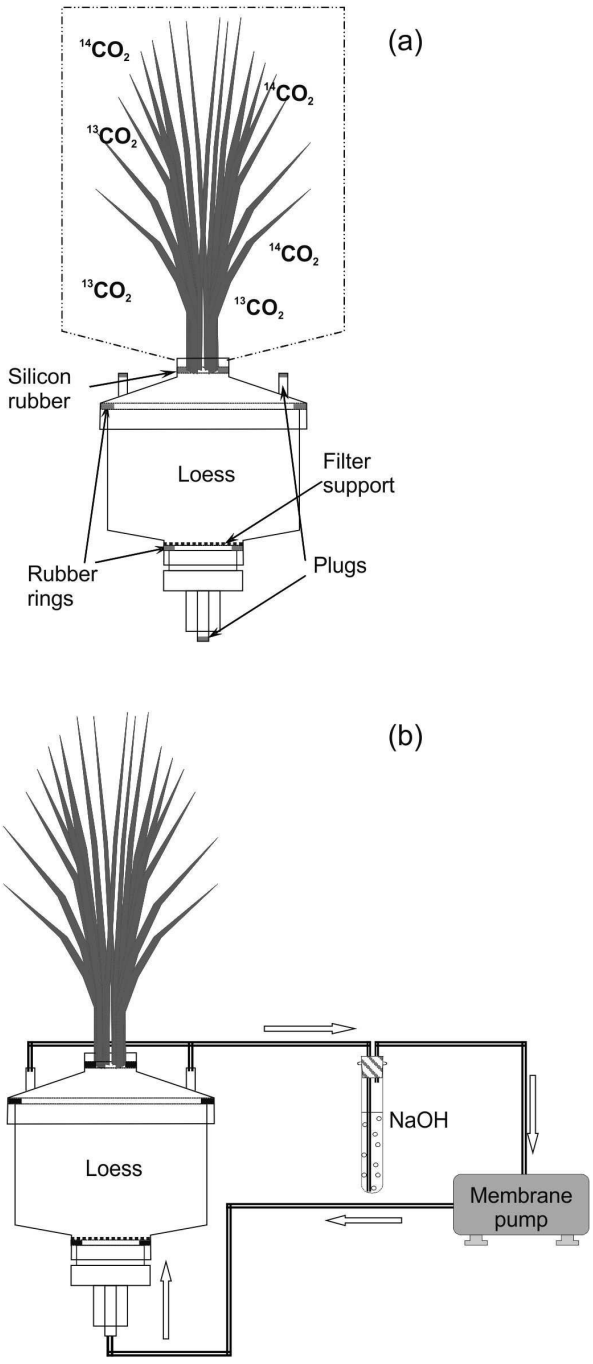

Fig. 2-1: Experimental setup. (a) Labeling of above-ground biomass in an airtight chamber with ^{13}C and ^{14}C labeled CO_2. (b) Trapping of CO_2 released by root and rhizomicrobial respiration in NaOH (modified after Kuzyakov and Siniakina, 2001).

To measure the amounts of C tracer incorporated into loess $CaCO_3$ by recrystallization, two grams (corresponding to 70 mg carbonatic C) of every dry loess sample were treated with 15 ml of 3 M H_3PO_4 in a closed system. Dissolution of samples by acid was chosen instead of combustion in order to release CO_2 only from $CaCO_3$ and not from organic compounds (root fragments, microbial remains, exudates). The CO_2 evolved from dissolution of $CaCO_3$ was trapped in 12 ml of NaOH to form Na_2CO_3. As the amount of dissolved $CaCO_3$ was known, an aliquot of the NaOH-Na_2CO_3-solution was

titrated (*Zibilske*, 1994) to test whether complete $CaCO_3$-C (irrespective if primary or secondary) of the dissolved loess sample was trapped as Na_2CO_3. This calculation could be applied because in our experiment, formation of secondary $CaCO_3$ in loess did not involve precipitation of additional carbonate but only recrystallization of already present loess $CaCO_3$, as the latter was the sole Ca^{2+} source.

For $\delta^{13}C$ analysis of loess carbonate, trapped CO_2 was precipitated as $SrCO_3$ by addition of 0.5 M $SrCl_2$ solution to the NaOH-Na_2CO_3-solution. No isotopic fractionation took place during precipitation because $SrCl_2$ solution was added in excess, and because of the low solubility product of $SrCO_3$ ($7 \cdot 10^{-10}$). The $SrCO_3$ precipitant was then purified by centrifugation and washing with deionised water as described by *Werth* and *Kuzyakov* (2008) and dried at 90 °C for 24 h. $SrCl_2$ was chosen for precipitation of CO_3^{2-} instead of commonly used $BaCl_2$, or $CaCl_2$, for the following reasons: Compared to $BaCO_3$, $SrCO_3$ requires lower temperature for thermal decomposition by $\delta^{13}C$ analyses on IRMS. At the same time, $SrCO_3$ has much lower solubility product than $CaCO_3$ – this ensures an absence of isotopic fractionation by complete precipitation of the dissolved CO_3^{2-}. $\delta^{13}C$ from loess $CaCO_3$ under plants and from unlabeled and unplanted loess samples was determined in $SrCO_3$ on an isotope ratio mass spectrometer (Delta Plus XL IRMS, Thermo Finnigan MAT, Bremen, Germany) connected to an elemental analyzer (EA 3000, Hekatech, Germany). $CaCO_3$ and acetanilide were used as reference materials for $\delta^{13}C$ measurement. Results are expressed in permil relative to the V-PDB reference standard, with an absolute precision of > 0.4‰.

To measure ^{14}C incorporated into loess carbonate by recrystallization, dissolution with H_3PO_4 and trapping of CO_2 in NaOH was repeated with 2 g loess (see above), and ^{14}C activity of loess carbonate was determined on 6 ml aliquots of NaOH mixed with scintillation cocktail (Rotiszint EcoPlus, Carl Roth, Germany) by an LS 6500 Multi-Purpose Scintillation Counter (Beckman, USA). The ^{14}C counting efficiency was at least 90%, the measurement error did not exceed 4%. The absolute ^{14}C activity was standardized by the H number method, using a ^{137}Cs external standard.

^{14}C activity of respired CO_2 trapped in NaOH was measured on 1 ml aliquots by a liquid scintillation counter (1450 LSC & Luminescence Counter MicroBeta TriLux, Perkin Elmer Inc., USA; ^{14}C counting efficiency $\geq$ 70%, measurement error $\leq$ 3.5%) which was standardized by SQP(E). Total carbon content of respired CO_2 trapped in NaOH was determined by titration (*Zibilske*, 1994).

2.3 Calculations

To calculate the amounts of C from respired CO_2 incorporated into loess carbonate and the initial rates of secondary carbonate formation, the amount of incorporated C tracer (^{13}C or ^{14}C) was referred to the amount of C tracer in respired CO_2-C. The only difference between ^{13}C and ^{14}C approach is that for the former, the atom percent excess (difference between labeled sample and natural abundance) was used. Concerning the ^{14}C approach, in contrast, ^{14}C specific activity was used for calculation. As natural ^{14}C content of unlabeled loess

$CaCO_3$, in terms of the used methodology, is zero, subtraction of ^{14}C natural abundance was not necessary.

For the approach with ^{13}C labeling, $\delta^{13}C$ values of $CaCO_3$ from all loess samples were converted into ^{13}C atomic percent (Equ. 1), where R is the $^{13}C/^{12}C$ ratio of the international PDB reference (R=0.0112372). Based on ^{13}C mass balance, the initial recrystallization rate was calculated as atom percent excess in labeled loess carbonate ($A_l^{CaCO3} - A_{NA}^{CaCO3}$), divided by atom percent excess in CO_2 respired by ^{13}C labeled plants ($A_l^{CO2} - A_{NA}^{CO2}$) and by the time (t) between the labeling and the sampling (Eq. 2).

$$A = 100 \bullet \frac{R \bullet (\frac{\delta^{13}C}{1000} + 1)}{1 + R \bullet (\frac{\delta^{13}C}{1000} + 1)} \quad (1)$$

$$CaCO_3 \text{ recrystallization rate } (^{13}C) = \frac{A_l^{CaCO3} - A_{NA}^{CaCO3}}{(A_l^{CO2} - A_{NA}^{CO2}) \bullet t} \quad (2)$$

For the second approach, the ^{14}C specific activity ($^{14}C_{SA}^{CO2}$) of CO_2 respired by roots and rhizomicrobial biomass and accumulated for 5 days was calculated as the ratio of ^{14}C activity ($^{14}C^{CO2}$) and total C content (C_t^{CO2}) in respired CO_2 (Eq. 3). Assuming that the ^{14}C specific activity of respired CO_2 equals the ^{14}C specific activity of the recrystallized part of the loess $CaCO_3$, the amount of recrystallized $CaCO_3$-C ($C_{t_{recryst}}^{CaCO3}$) was calculated using the ^{14}C activity of loess $CaCO_3$ ($^{14}C^{CaCO3}$) (Eq. 4). The amount of recrystallized $CaCO_3$-C was divided by the total $CaCO_3$-C content of the loess (C_t^{CaCO3}) and by the time (t) between labeling and sampling (5 days), yielding the initial carbonate recrystallization rate (Eq. 5).

$$^{14}C_{SA}^{CO2} = \frac{^{14}C^{CO2}}{C_t^{CO2}} \quad (3)$$

$$C_{t_{recryst}}^{CaCO3} = \frac{^{14}C^{CaCO3}}{^{14}C_{SA}^{CO2}} \quad (4)$$

$$CaCO_3 \text{ recrystallization rate } (^{14}C) = \frac{C_{t_{recryst}}^{CaCO3}}{C_t^{CaCO3} \bullet t} \quad (5)$$

Standard errors of means (SEM) are presented in the figures.

3 Results

3.1 Recrystallization rates

^{14}C analyses showed that more than 99% of the applied $^{14}CO_2$ label was assimilated by the plants during the labeling procedure (*Gocke* et al., 2010a). As both tracers were applied

simultaneously, and as isotopic preference by the plants during assimilation of labeled CO_2 is negligible, we also assume near complete assimilation of the ^{13}C label.

$\delta^{13}C$ values of loess carbonate were -1.19 ± 0.09‰ for unlabeled and unplanted loess, -1.06 ± 0.08‰ for wheat-planted and -1.37 ± 0.18‰ for ryegrass-planted loess (Fig. 2-2a). The ^{13}C atom percent excess in $CaCO_3$ from rhizosphere loess planted and labeled with wheat revealed a portion of recrystallized $CaCO_3$ of 0.032 ± 0.020% of total loess carbonate after 5 days (Fig. 2-2a). This corresponds to a mean recrystallization rate of $6.35 \cdot 10^{-5}$ day^{-1} (Table 2-1). For ryegrass, amounts of recrystallized $CaCO_3$, and consequently the recrystallization rates, could not be determined because the respective $\delta^{13}C$ values were not significantly different from the initial ^{13}C abundance (Fig. 2-2a).

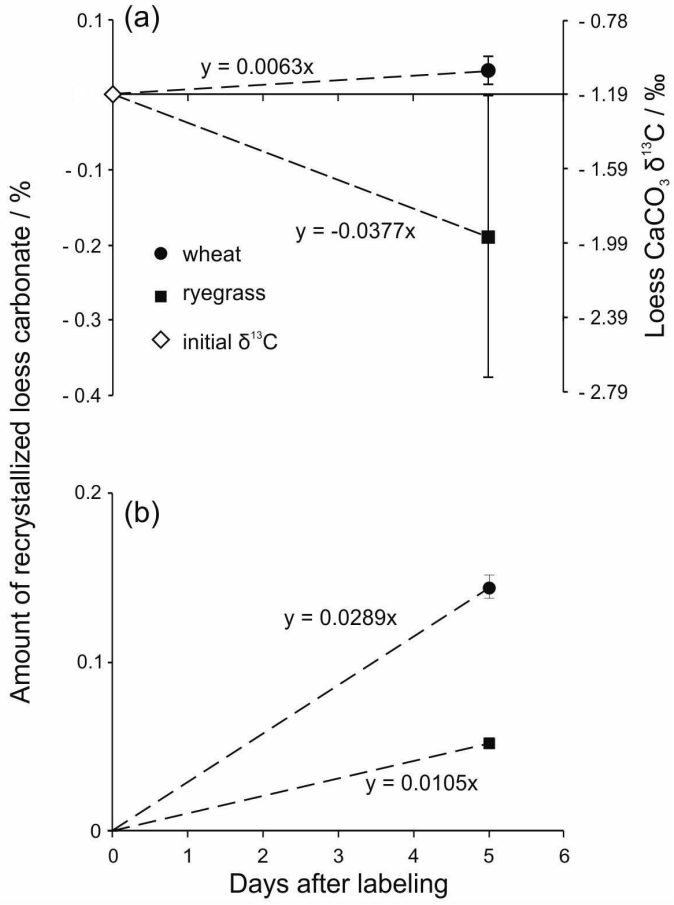

Fig. 2-2: Amounts of recrystallized carbonate (± SE) as a percentage of total loess carbonate five days after the labeling, based either on the (a) ^{13}C or (b) ^{14}C labeling approach. For the former, $\delta^{13}C$ values of loess $CaCO_3$ are presented on the right Y-axes. The diagrams show the amounts of carbonate recrystallized after labeling, irrespective of prior recrystallization.

Based on the ^{14}C activity in loess $CaCO_3$ and the ^{14}C specific activity of CO_2 evolved by root and rhizomicrobial respiration, we calculated the amount of loess carbonate recrystallized within 5 days. After 5 days, the amount of recrystallized carbonate (as a portion of the total loess carbonate) was 0.144 ± 0.007% for wheat and 0.052 ± 0.003% for ryegrass (Fig. 2-2b).

These amounts correspond to mean rates of $2.89 \cdot 10^{-4}$ day^{-1} and $1.05 \cdot 10^{-4}$ day^{-1} under wheat and ryegrass, respectively (Tab. 2-1).

Table 2-1

$CaCO_3$ recrystallization rates in rhizosphere and non-rhizosphere (only ^{14}C) loess calculated based on ^{13}C and ^{14}C labeling, derived from loess planted with wheat and ryegrass. For ryegrass, the ^{13}C approach did not provide reasonable results, which is also reflected in Fig. 2-2. For comparison, ranges of recrystallization rates without plants under CO_2 concentrations between 380 and 50000 ppm in loess air (Gocke et al., 2010b) are also displayed.

	Wheat rhizosphere (non-rhizosphere)	Ryegrass rhizosphere (non-rhizosphere)	Without plants (*Gocke* et al., 2010b)
Isotopic approach	$CaCO_3$ recrystallization rates /day^{-1}		
^{13}C	$6.35\ [\pm 4.00] \cdot 10^{-5}$	$-\ 3.77\ [\pm 3.74] \cdot 10^{-4}$	$0.3 \cdot 10^{-5} - 1.4 \cdot 10^{-5}$
^{14}C	$2.89\ [\pm 0.13] \cdot 10^{-4}$ ($1.19\ [\pm 0.02] \cdot 10^{-4}$)	$1.05\ [\pm 0.06] \cdot 10^{-4}$ ($4.52\ [\pm 0.07] \cdot 10^{-5}$)	$0.4 \cdot 10^{-6} - 1.7 \cdot 10^{-6}$

Over long periods (hundreds to thousands of years), the amount of primary $CaCO_3$ exchanged with $^{14}CO_2$ of rhizosphere respiration can be described by an exponential curve (1st order kinetics). During the first months of plant growth, however, the amount of recrystallized loess carbonate increases nearly linearly due to very low rates (*Kuzyakov* et al., 2006). Therefore, the slopes of the trend curves (Fig. 2-2) correspond to the initial recrystallization rates.

3.2 Periods of $CaCO_3$ recrystallization

Based on the initial rates, periods necessary for complete recrystallization of primary loess carbonate were calculated. Assuming that not only the primary loess $CaCO_3$, but also secondary $CaCO_3$ is recrystallized with CO_2 released by root and rhizomicrobial respiration, the increase of the amount of recrystallized carbonate is described by an exponential approach (Eq. 6). As high CO_2 concentration in soil is maintained predominantly during the growth period by root and rhizomicrobial respiration, typical growing seasons of vegetation (4 months for wheat, 6 months for ryegrass) were considered in Eq. 6. The amount of recrystallized carbonate ($CaCO_3(t)$) was calculated as follows:

$$CaCO_3(t) = 100 \bullet (1 - e^{-t \bullet rate \bullet \frac{GS}{365}}) \qquad (6)$$

with t: time in years, rate: recrystallization rate in day^{-1}, GS: growing season in days per year.

Applying this approach to the rates based on ^{13}C, 99% recrystallization of primary loess carbonate requires 590 years for wheat. Extrapolation of the values from ^{14}C labeling yielded shorter recrystallization periods of 130 and 240 years for wheat and ryegrass, respectively (Fig. 2-3, Tab. 2-2).

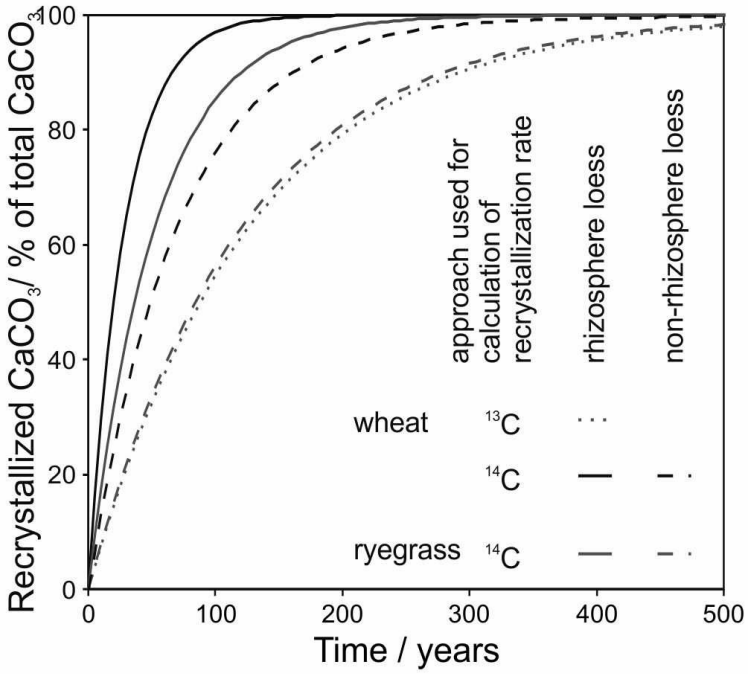

Fig. 2-3: $CaCO_3$ recrystallization periods modeled for rhizosphere loess (continuous lines) based on recrystallization rates estimated by isotopic exchange with ^{13}C (only wheat) or ^{14}C. For comparison, recrystallization periods for non-rhizosphere loess (dashed lines) based on the ^{14}C approach (for values of recrystallization rates see Tab. 2-1) are also displayed in the diagramm. Please note that all data were derived from sealed plant pots where recrystallization of loess $CaCO_3$ takes place faster than under natural conditions.

Table 2-2

Periods necessary for 99% recrystallization of rhizosphere loess $CaCO_3$, calculated based on ^{13}C and ^{14}C labeling. Growing seasons of 4 and 6 months were assumed for wheat and ryegrass, respectively. Data in brackets give the lower and upper limit of the recrystallization periods, based on upper and lower limit of recrystallization rates.

	Wheat rhizosphere	Ryegrass rhizosphere
Isotopic approach	$CaCO_3$ recrystallization peridos /years	
^{13}C	590 [365 – 1600]	n.d.
^{14}C	130 [125 – 140]	240 [225 – 255]

4 Discussion

4.1 Isotopic pulse labeling

Based on the exchange of primary loess $CaCO_3$-C with CO_2 from root and rhizomicrobial respiration, we used the isotopic exchange to estimate the amount of recrystallized $CaCO_3$ in loess. ^{13}C and ^{14}C isotopes were employed simultaneously as tracers to test their feasibility for assessing the very slow carbonate recrystallization process.

Due to increased CO_2 partial pressure (CO_2 accumulation within the sealed plant vessels between labeling and sampling), we assume higher recrystallization rates in our experiment than under field conditions. Sealing the plant pots was necessary to determine the ^{14}C specific

activity of CO_2 released by root and rhizomicrobial respiration, which in turn was used to calculate the recrystallization rate based on the ^{14}C approach. The very low amounts of recrystallized carbonate in loess (maximum 0.14%, Fig. 2-2) require a very sensitive method for estimation of recrystallization rates during short periods such as in our study. Even small differences in the rate entail huge variations concerning the modeled periods necessary for complete recrystallization of primary carbonate and formation of secondary carbonate.

4.2 Estimated $CaCO_3$ recrystallization rates

In contrast to previous studies (*Kuzyakov* et al., 2006, *Gocke* et al., 2010a), recrystallization rates were not estimated over time intervals of several weeks after multiple pulse labeling, but after application of one isotopic pulse. This might entail uncertainties regarding precision of the estimated rates. However, all previous recrystallization studies demonstrated that constant CO_2 supply during the initial stage (weeks – months) of plant growth leads to linear increase of recrystallized $CaCO_3$. Thus, slopes of Fig. 2-2 correspond to initial recrystallization rates in loess, and can be used as approximate values for comparison of ^{13}C and ^{14}C results.

The results based on ^{14}C labeling showed that the methodological sensitivity of the ^{14}C approach is high enough to detect process rates as slow as $CaCO_3$ recrystallization in plant experiments. The ^{14}C approach yielded rates in the same order of magnitude for both plant species (wheat: $2.89 \cdot 10^{-4}$ day^{-1}, ryegrass: $1.05 \cdot 10^{-4}$ day^{-1}), while the ^{13}C approach produced usable results only for wheat ($6.35 \cdot 10^{-5}$ day^{-1}). For ryegrass, no accumulation of ^{13}C in loess $CaCO_3$ by carbonate alteration was found. At least for wheat, both approaches showed that labeled C was incorporated into the loess carbonate by recrystallization. The resulting rates (^{13}C versus ^{14}C approach) differed from each other concerning mean value and in particular standard errors of means between the replications, which were much higher for results based on ^{13}C (up to ± 100% of the mean) compared to that based on ^{14}C (max. ± 6% of the mean, Fig. 2-2, Tab. 2-1).

4.3 Precision of ^{13}C and ^{14}C approaches

The recrystallization rate based on ^{13}C incorporation in $CaCO_3$ in the loess close to the root surface (rhizosphere) was one order of magnitude lower than rates based on ^{14}C incorporation and showed much higher standard errors (Tab. 2-1). We therefore did not analyze $\delta^{13}C$ in non-rhizosphere loess carbonate because we assumed even less reliable values there. In contrast, the ^{14}C approach enabled the plant-derived C incorporated into secondary carbonate to be determined even in loess not adjacent to roots (*Gocke* et al., 2010a): rhizosphere processes therefore clearly play an important role in secondary carbonate formation. The importance of roots and rhizosphere is obvious by consideration of rhizolith forms and formation processes (*Lambers* et al., 2009 and references therein). We presume that the ^{13}C approach will not work for non-rhizosphere loess carbonate because of insufficient sensitivity. There are two reasons for this lower sensitivity. First, the theoretical detection limit of ^{13}C mass spectrometry is six orders of magnitude less (10^{-7} mol) than that of ^{14}C liquid scintillation counting (10^{-13} mol). Second, in case of ^{13}C labeling, the ^{13}C is already

present in $CaCO_3$ of unplanted and unlabeled loess. Although the ^{13}C content is increased by labeling loess carbonate, the amount of ^{13}C incorporated remains very small due to the very low recrystallization rates (even after periods longer than in our study). Therefore, the amount of ^{13}C incorporated in the carbonate is still extremely low compared to that already present in loess. Accordingly, analyses of $\delta^{13}C$ near the level of natural abundance depend strongly on measurement accuracy. This problem does not exist in ^{14}C labeling: the age of the Nussloch loess-paleosol sequence lies within the last glacial-interglacial cycle (ca. 20 – 120 ka bp, *Antoine* et al., 2001), and the used loess originated from a depth of 15 m below the present surface. The natural ^{14}C content in the loess $CaCO_3$ is therefore zero.

Despite careful sampling and sample preparation (mixing of loess samples, dissolution of $CaCO_3$, reprecipitation as $SrCO_3$, washing and centrifugation), a variation of 1 – 2‰ between replications of the same treatment can occur due to inhomogeneous distribution of ^{13}C incorporated into $CaCO_3$. Because of high $\delta^{13}C$ background, even smaller variation, as observed in our experiment, led to differences in the estimated recrystallization rates of up to one order of magnitude (Tab. 2-1).

Recalculation of the hypothetical increase of $CaCO_3$-$\delta^{13}C$ values based on ^{14}C data (Tab. 2-1 and Fig. 2-2b) yielded very small changes of the initial $\delta^{13}C$ of loess $CaCO_3$ (0.24‰ for wheat and 0.04‰ for ryegrass). These changes are too low for reliable $\delta^{13}C$ analysis. Therefore, we strongly recommend application of ^{14}C tracer for estimation of initial $CaCO_3$ recrystallization rates. Accordingly, the isotopic exchange based on ^{14}C is probably the only possibility to estimate such slow processes rates.

As shown in this study, the recrystallization rate based on the ^{13}C approach could be calculated only for wheat plants, which received twice as much ^{13}C (10 mg per plant pot) as ryegrass plants (5 mg per plant pot). One potential way to bypass the low sensitivity of ^{13}C labeling might be to increase the amounts of ^{13}C applied, boosting the percentage of ^{13}C applied for the pulse, thus leading to a higher percentage of ^{13}C recovered in secondary carbonate. This, however, might entail methodological difficulties (overpressure in the labeling chamber by the high amount of released CO_2, potentially incomplete assimilation by plants because of CO_2 oversupply). It might also lead to unnatural partitioning of assimilates due to very high CO_2 content in the chamber. In contrast, the CO_2 concentration in the chamber is increased only marginally when applying ^{14}C because the mass of ^{14}C necessary to estimate the recrystallization rate is negligibly low (µg).

4.4 Reproducibility and reliability of recrystallization rates, and further advantages of the ^{14}C approach

Compared with literature data (*Kuzyakov* et al., 2006), the ^{14}C approach showed high reproducibility of rates (10^{-5} – 10^{-4} day^{-1} for non-rhizosphere loess, 10^{-4} day^{-1} for rhizosphere loess). In the ^{13}C approach, lower sensitivity, high standard errors of means between replicates of rhizosphere loess samples, and results only for one of the two plant species suggest that it is not possible to estimate the recrystallization rate in loess not adjacent to roots. ^{14}C isotopic exchange clearly yields more dependable results.

One further indicator of the better reliability of ^{14}C over ^{13}C is the fact that, without plants, the rates calculated based on ^{13}C (10^{-5} day^{-1}) were higher than when using ^{14}C (10^{-6} day^{-1}) (*Gocke* et al., 2010b), while the situation was *vice versa* in the current study with rhizosphere loess (10^{-5} day^{-1} for ^{13}C and 10^{-4} day^{-1} for ^{14}C) (Tab. 2-1). Carbonate recrystallization rates in planted loess should always be higher than in unplanted loess and even more so when comparing unplanted loess and rhizosphere loess. This leads to implausible ^{13}C rates.

Finally, when quantifying pedogenic carbonate recrystallization, it might be interesting to quantify the tracer also in the carbon remaining in water after washing the loess (dissolved inorganic and organic carbon, DIC, DOC) to better understand soil carbonate dissolution and recrystallization. In the rhizosphere, root-derived C (exudates and their microbial metabolites, DOC) is rapidly microbially decomposed to CO_2 (*Fischer* et al., 2010). Moreover, the CO_2 evolved from root and rhizomicrobial respiration and dissolved as HCO_3^- (DIC) directly contributes to the isotopic re-equilibration with primary carbonate (*Cerling*, 1984, *Nordt* et al., 1996). The added label in these dissolved C pools can be traced by IRMS ($\delta^{13}C$) or by ^{14}C liquid scintillation counting of DIC and DOC solution. In many cases, however, it is easier and more convenient to estimate the kinetics of the isotopic exchange from DIC by ^{14}C than by ^{13}C analysis.

4.5 Plausibility of modeled recrystallization periods

Extrapolation of initial rates for long periods bears some uncertanties, partly connected to the fact that the initial rates may not correspond to the later rates during soil development. As there are not any other approaches available, in previous studies we showed the possible range of recrystallization periods based on alternative assumptions, e.g. length of growing season and formation of carbonate concretions (*Kuzyakov* et al., 2006).

The length of the modeled recrystallization period strongly depended on the isotope applied for labeling, and thus on the precision of the method. By extrapolating the initial rate based on ^{13}C labeling, 590 years were necessary for 99% recrystallization of primary loess carbonate, while the ^{14}C approach yielded a maximum value of 240 years (Fig. 2-3), with a narrow range between 225 and 255 years (Tab. 2-2). Taking into account the upper and lower limit of the ^{13}C based rate (Tab. 2-1), however, the 99% recrystallization period based on ^{13}C data varies between 365 and 1600 years (Tab. 2-2). The ^{14}C data also showed that the rates in loess not adjacent to roots are approximately half that in rhizosphere loess (Tab. 2-1), yielding recrystallization periods of 315 and 555 years for wheat and ryegrass, respectively, in non-rhizosphere loess (Fig. 2-3).

Due to the uncertainties caused from the experimental design with one isotopic pulse and sampling 5 days afterwards, these calculated recrystallization periods have to be regarded as an approximation. For this reason, calculated values were compared to ages of natural pedogenic carbonates from literature, which, however, are rare because of uncertainties for radiocarbon dating of pedogenic carbonates (*Bowler* and *Polach*, 1971, *Amundson* et al., 1994).

In terms of magnitude, radiocarbon ages of inorganic carbon measured in soils of known ages support our estimations under controlled conditions. In general, radiocarbon ages from pedogenic carbonates in semiarid regions are in a magnitude of $n \cdot 10^3$ years (*Becker-Heidmann* et al., 1996). Under semi-arid climatic conditions, the ^{14}C age of $CaCO_3$ indicated that carbonate whose total content in a soil is up to 2.5% can be completely recrystallized within 1 – 3.8 ka (*Pendall* et al., 1994). *Pustovoytov* and *Leisten* (2002) demonstrated that after a 1,000-year-long exposure of artificial lime mortar to soil weathering under Mediterranean climate, 10% of the initial carbonate was recrystallized in the upper 20 cm of soil. In this case, full recrystallization would probably take tens of thousands of years. Note, however, that this time is required for a complete recrystallization of artificial mortar, which is a relatively dense material with a substantially higher $CaCO_3$ content than in loess. For more loose substrates with lower carbonate content, as in the case of loess, the rates are presumably higher, leading to shorter recrystallization periods.

Specifically for loesses, we are unaware of any work directly showing carbonate recrystallization rates in natural profiles. However, the ^{14}C ages of secondary carbonate accumulations (calcified root cells) can be younger than the ages of the loess itself. In a central European loess-paleosol section, the ^{14}C ages of secondary carbonates at 0.6 – 3 m depth were ca. 6 – 9 ka BP (*Pustovoytov* and *Terhorst*, 2004), whereas the loess accumulation in this area ceased in the Late Pleistocene (ca. 16 ka bp, *Antoine* et al., 2001). These data imply that measurable neoformation of carbonate in loesses can take place even at depth on the Holocene time scale, which further suggests potential recrystallization of already formed carbonate.

The above mentioned recrystallization periods, calculated on the basis of the age of soil formation, are longer than our modeled recrystallization periods, especially those calculated based on the ^{14}C approach. We explain this first by the fact that we compared values for recrystallization rates from rhizosphere, where rates can be up to twice as high as in non-rhizosphere loess, leading to considerably shorter recrystallization periods (*Gocke* et al., 2010a). These conditions are, however, restricted to few mm around the plant roots. For a substantial part of the soil, lower recrystallization rates and therefore longer recrystallization periods than in the rhizosphere can be assumed. Second, the properties of the primary carbonate are an important criterion. In contrast to artificial mortar, primary carbonate in our study was homogeneously disseminated as small crystals (size: tens of µm) and constituted 29.0% of the loess. Third, in our study, high CO_2 concentrations in loess due to sealing of the plant pots probably led to enhanced dissolution of loess $CaCO_3$ and precipitation of secondary $CaCO_3$, resulting in overestimation of initial recrystallization rates and shorter recrystallization periods when compared to field conditions. It appears likely that one or more of these factors led to underestimation of recrystallization periods in our experiment. Therefore we assume that modeled data based on the ^{14}C approach better fit with radiocarbon ages measured on carbonate materials from soil profiles of known ages.

5 Conclusions

Assessing very slow $CaCO_3$ recrystallization rates over short periods requires a very sensitive and precise method. Based on the isotopic exchange between primary loess carbonate and C from respired CO_2, we calculated initial rates by determining the amount of C incorporated into secondary carbonate from respired CO_2 of dual ^{13}C and ^{14}C pulse labeled plants.

We showed that very small portions of primary loess carbonate were recrystallized in the rhizosphere, leading to rates of 10^{-5} day^{-1} (^{13}C approach) and 10^{-4} day^{-1} (^{14}C approach). Extrapolating the rate estimated by ^{13}C labeling to longer periods indicates that about 600 (365 – 1600) years are required for complete recrystallization of primary carbonate, however this approach was connected with very high standard errors. In contrast, the ^{14}C labeling showed sufficiently higher precision and reproducibility and indicated full recrystallization periods of 130 (125 – 140) or 240 (225 – 255) years. Therefore, the ^{14}C approach is recommended as a preferential tool to estimate recrystallization rates of pedogenic carbonates.

Estimated initial recrystallization rates and periods have to be regarded as an approximation, because precision is limited by the short experiment duration. Radiocarbon dates on carbonates from soil profiles with known ages in semi-arid environments suggest that a complete cycle of carbonate recrystallization requires $n \cdot 10^3$ years. Taking into account the slower recrystallization in non-rhizosphere, this supports our estimations under controlled conditions.

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Study 3: Carbonate recrystallization in root-free soil and rhizosphere of *Triticum aestivum* and *Lolium perenne* estimated by ^{14}C labeling

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Abstract

Under arid and semiarid conditions, pedogenic (secondary) carbonates are formed in soil by precipitation of Ca^{2+} from soil parent material with dissolved CO_2 originating from root and rhizomicrobial respiration. $\delta^{13}C$ values of secondary $CaCO_3$ record the photosynthetic pathway of former vegetation and is therefore used as a tool for paleoenvironmental studies. The time scale of pedogenic carbonate formation as well as the influence of several environmental factors are crucial, yet poorly known. We estimated the recrystallization rate of pedogenic carbonate by the ^{14}C isotopic exchange method. $^{14}CO_2$ was assimilated by plants, respired into the rhizosphere and subsequently incorporated into secondary carbonate by recrystallization of primary loess carbonate. With ascending number of $^{14}CO_2$ pulses, the amount of rhizosphere ^{14}C recovered in loess $CaCO_3$ increased linearly, leading to recrystallization rates of $3.2 \cdot 10^{-5}$ and $2.8 \cdot 10^{-5}$ day^{-1} for wheat and ryegrass, respectively. In loess close to roots, recrystallization rates more than twice as high were obtained. Extrapolating these rates we showed that several hundred years are necessary for complete recrystallization of primary loess $CaCO_3$ in root-free substrate, assuming that both primary and secondary carbonate is recrystallized several times. In contrast, the process probably takes only decades in rhizosphere loess if carbonaceous encrustations form around the root, impeding repeated recrystallization. This indicates the importance of rhizosphere processes (e.g. respiration of roots and microorganisms, exudation) for secondary carbonate formation.

Keywords: ^{14}C pulse labeling; loess; pedogenic $CaCO_3$; recrystallization rate; rhizosphere processes; soil CO_2

1 Introduction

The formation of pedogenic (secondary) carbonates by interaction of Ca^{2+} from soil parent material with dissolved CO_2 of soil air, plays an important role in pedogenesis in soils of semiarid and arid regions like southwest USA (Machette 1985) or Russian steppe (Lebedeva et al. 2002, Lebedeva & Ovechkin 2003). Highest abundance of secondary carbonate accumulations occurs under semiarid conditions with mean annual precipitation of less than approximately 500 mm (Birkeland 1999).

Stable carbon (C) isotope studies demonstrate that C of pedogenic carbonates originates from root and rhizomicrobial respiration (Cerling 1984, Cerling et al. 1989). C from primary (lithogenic) $CaCO_3$, if the latter is present, is replaced by C from soil CO_2 and thus does not contribute to the C isotopic composition of secondary carbonates (Cerling 1984, Nordt et al. 1996). The isotopic composition ($\delta^{13}C$ and $\Delta^{14}C$) of pedogenic carbonate is thus related to the isotopic composition of soil CO_2; it reflects the former local vegetation with regard to the photosynthetic pathway (Cerling et al. 1989, Amundson et al. 1989, Cerling & Quade 1993). Based on the C exchange during recrystallization, pedogenic carbonates can help reconstruct the paleoclimate and paleovegetation (e.g. Buck & Monger 1999; Deutz et al. 2001, Boguckyi et al. 2006, Achyuthan et al. 2007, Pustovoytov et al. 2007). They can also help estimate former CO_2 concentrations in the Earth's atmosphere (Cerling 1991, 1992; Royer et al. 2001;

Tanner et al. 2001) and are useful in chronological studies using radiocarbon dating (e.g. Amundson et al. 1994, Wang et al. 1996, Pustovoytov et al. 2007). Beyond stable carbon isotope composition, also the $^{18}O/^{16}O$ ratio in pedogenic carbonate can provide paleoclimatic information (e.g. Cerling 1984, Dworkin et al. 2005). Royer et al. (2001), however, pointed out that the temporal resolution of stable isotope analysis based on pedogenic $CaCO_3$ is limited by the time scale of secondary carbonate formation.

The high potential of pedogenic carbonate as a proxy is complicated by the fact that carbonate, once formed, theoretically can be recrystallized with time and thus lose its paleonvironmental and chronological value. This potential problem of alteration of carbonate archives by diagenesis or overprint has been recognized in the literature (Cerling 1991, Amundson et al. 1994, Budd et al. 2002). Changes in profile distribution of ^{14}C ages of artificial lime mortar have been demonstrated for a soil on a cultural layer (Pustovoytov & Leisten, 2002).

Few attempts, however, have been made to estimate the recrystallization period of pedogenic $CaCO_3$ in the field, based either on radiocarbon data or on stable carbon isotopic composition. Pendall et al. (1994) estimated the recrystallization rate indirectly by comparing radiocarbon ages of these carbonates with ages determined independently from isotopic methods. Very low recrystallization rates impede assessing the process and rule out applying stable carbon isotope natural abundance. Thus, current methods of geosciences are insufficient to determine the recrystallization rate of secondary carbonates in situ.

A new approach – the estimation of pedogenic carbonate recrystallization rates under controlled conditions by artificial ^{14}C isotopic labeling – was introduced by Kuzyakov et al. (2006). The method enables estimating the amount of root-derived C incorporated into primary $CaCO_3$ by recrystallization by isotopic exchange. It involves exposing plants to an artificially labeled $^{14}CO_2$ atmosphere. After $^{14}CO_2$ assimilation by plants, transport of assimilates belowground, subsequent release into the rhizosphere by root and rhizomicrobial respiration and recrystallization with primary loess carbonate, the ^{14}C label was recovered quantitatively in loess $CaCO_3$.

Our previous study (Gocke et al. 2010) showed that recrystallization of loess carbonate by direct contact with added CO_2 takes place also without plants, but in a smaller scope. The estimated rates of 10^{-7} day^{-1} without plants (Gocke et al. 2010) were approximately two orders of magnitude lower than rates with plants (Kuzyakov et al. 2006). This indicates the importance of biological processes (root respiration and microbial decomposition of root exudates) for the recrystallization of soil carbonate. This calls for considering the effect of living plants on the carbonate recrystallization rate. As no other studies have estimated this rate, we compared the effect of two plants: wheat and ryegrass.

This study aims: (1) to demonstrate the methodological sensitivity and reproducibility of the ^{14}C isotopic exchange method for quantifying pedogenic carbonate recrystallization, (2) to estimate initial recrystallization rates of loess $CaCO_3$ under controlled conditions and to extrapolate the periods necessary for complete recrystallization to show the accuracy limit of temporal resolution in paleoenvironmental studies based on $\delta^{13}C$ values of pedogenic

carbonates, (3) to uncover possible differences in recrystallization intensities between agricultural cereals and pasture plants, (4) to reveal the influence of root vicinity on $CaCO_3$ recrystallization.

2 Material and methods

2.1 Plants and growing substrate

To test the effect of plant species on the recrystallization rate of pedogenic carbonate, two species – the agricultural cereal winter wheat [*Triticum aestivum* (L.)] and pasture grass [perennial ryegrass, *Lolium perenne* (L.)] – were investigated. Both species belong to the family Poaceae, but show differences concerning growth rate, percentage of assimilated C that the plant allocates belowground and invests into rhizosphere processes, and length of the growing season.

The plants were grown on loess originating from a depth of 15 m below the present soil surface. Loess from this depth is not influenced by modern pedogenic processes like humification, leaching or calcification. For our experiment this means that, firstly, CO_2 concentration and fluxes from root and rhizomicrobial respiration are not disturbed by CO_2 produced by microbial decomposition of soil organic matter and, secondly, the loess $CaCO_3$ is primary, i.e. not recrystallized, and shows a uniform spatial distribution and grain size. In summary, we simulated the conditions of initial soil formation in loess.

2.2 Experiment layout and plant growing conditions

Wheat and ryegrass were grown on loess from an open cast mine at Nussloch (SW Germany, see Bente & Löscher 1987). The loess contained 29% $CaCO_3$. Many studies about the loess from Nussloch contain sedimentological (e.g. Löscher & Zöller 2001) as well as stratigraphic and paleoclimatic (e.g. Hatté et al. 1999, Moine et al. 2005) information.

As plant pots, we used polycarbonate filtration devices with three inlets in the lid and one main opening for growth of the plant shoots (CombiSart, Sartorius AG, Germany; real volume 340 ml). The devices were described in detail by Kuzyakov & Siniakina (2001). The chamber of the CombiSart device was used as a loess-root compartment, separated from the outlet in the bottom by a perforated filter support overlain by a viscose mesh. The CombiSart devices can be unscrewed, allowing non-destructive sampling of loess and roots.

Seeds from wheat and ryegrass were pre-germinated for 3 and 6 days, respectively. Each plant pot was filled with 450 g of air-dried and sieved loess and planted with 4 seeds of wheat or 6 seeds of ryegrass. For the introduction of microorganisms, 10 ml of soil extract from a Haplic Luvisol (developed from loess) were added. As loess, contrary to soil, does not contain nutrients, the plants were treated with Hoagland nutrient solution (Hoagland & Arnon 1950). The nutrient solution was modified by doubling the amounts of KH_2PO_4 and KNO_3 and omitting the $Ca(NO_3)_2 \cdot 4\ H_2O$ to compensate the huge amount of Ca^{2+} ions available from loess $CaCO_3$. The applied nutrient solution contained 138, 62 and 469 $\mu g\ ml^{-1}$ of N, P and K, respectively. The plants were grown at 14/10 h day/night periods, light intensity of 300 µmol

m^{-2} s^{-1}, and loess moisture was equal to 70% of water holding capacity (WHC = 28% of loess weight).

The experiment layout included seven treatments for each plant species:

1) Plants in sealed pots, labeled once
2) Plants in sealed pots, labeled twice
3) Plants in sealed pots, labeled three times
4) Plants in sealed pots, labeled four times
5) Plants in sealed pots, labeled five times
6) Plants in open pots, labeled twice
7) Plants in open pots, labeled five times.

For each of the seven treatments, three replications were made, yielding a total of 42 plant pots (21 pots for each plant). The first pulse labeling of wheat was done 27 days after planting, and of ryegrass 59 days after planting. The subsequent isotopic pulses were applied in intervals of five days.

2.3 ^{14}C labeling and sampling

One day before the first labeling, the openings in the pots were completely closed by plugs and sealed around the plant shoot. For this purpose, every shoot was encased with cellulose, covered by a non-toxic two-component silicone paste (NG 3170, Thauer & Co., Germany). The seal was tested for air leaks. By using sealed plant pots, the air from the root-loess compartment could be separated from the atmospheric air, thus avoiding a loss of ^{14}C labeled and total CO_2. The two treatments with unsealed pots were applied to simulate natural conditions by allowing air exchange between atmosphere and the loess-root compartment. Directly before applying the first ^{14}C pulse label, every pot was flushed with atmospheric air for several hours by a membrane pump (Type SMG4, Gardner Denver Thomas GmbH, Germany). All CO_2 previously released by root and rhizomicrobial respiration and accumulated within the rhizosphere during plant growth was thus removed from the pots. The moisture in loess was then enhanced to 95% of WHC to bypass dryness during the five days until the next ^{14}C pulse.

All pots of one plant species were labeled simultaneously in an airtight chamber. The label, consisting of 407 kBq of ^{14}C as $Na_2{}^{14}CO_3$ (ARC Inc., USA) per plant pot, was diluted with 10 ml of de-ionized water in a 30 ml vial. Previously, the water was slightly alkalinized to prevent loss of ^{14}C activity by exchange with atmospheric CO_2. After connecting the output of the label solution vial with the label chamber, $^{14}CO_2$ was released by adding 3 ml of 5 M H_2SO_4 to the label solution and pumped through the chamber in a closed cycle for 10 min by a membrane pump. The plants stayed in the chamber for 3 h (wheat) or 4 h (ryegrass) to allow for assimilation of the ^{14}C labeled CO_2. After that time, the air of the chamber was pumped through 15 ml of 1 M NaOH to trap the remaining unassimilated CO_2. The chamber was opened and the plants continued growth under normal conditions.

Prior to the next labeling, the rhizosphere air of every plant pot was pumped through 15 ml of 1 M NaOH for 90 min in a closed cycle to trap CO_2 from root and rhizomicrobial respiration. Three plants were selected for analysis and the remaining plants were labeled again as described above.

From the harvested replications, shoots were cut and roots were separated from loess ("non-rhizosphere loess") with tweezers. The roots, together with adhering loess (in the following termed "rhizosphere loess"), were washed with 60 ml of slightly alkalinized de-ionized water to remove loess and dissolved inorganic and organic carbon (DIC and DOC). Both shoots and roots were dried at 60 °C and grinded in a ball mill (MM200, Retsch, Germany). The solution obtained from root washing, containing loess directly adjoining the roots, was filtrated by a stainless steel pressure filter holder (SM 16249, Sartorius, Germany). Three grams from mixed non-rhizosphere loess were washed with 10 ml of slightly alkalinized de-ionized water. All loess samples were dried at 90 °C.

2.4 ^{14}C sample analysis

After the labeling, ^{14}C activities of the residue of the label $Na_2{}^{14}CO_3$ solution and of unassimilated CO_2 trapped in NaOH were measured on 1 ml aliquots mixed with 2 ml of scintillation cocktail (Rotiszint EcoPlus, Carl Roth, Germany) after decay of chemiluminescence (for NaOH). The ^{14}C measurements were done using a 1450 LSC & Luminescence Counter (MicroBeta TriLux, Perkin Elmer Inc., USA). The ^{14}C counting efficiency was at least 70%, the measurement error did not exceed 3.5%. The absolute ^{14}C activity was standardized by SQP(E) by adding increasing amounts of NaOH as a quencher. ^{14}C activity of respired CO_2 trapped in 15 ml of NaOH was measured on 1 ml aliquots in the same way.

To analyze ^{14}C incorporated into plant biomass, small amounts of plant material (35 mg from shoots and 45 mg from roots) were combusted in an oven ("Feststoffmodul 1300", AnalytikJena, Germany). The CO_2 released by combustion was trapped in 8 ml of 1 M NaOH. ^{14}C activity of the NaOH was measured on 1 ml aliquots as described above.

^{14}C activities of loess carbonate, DIC and DOC were measured on larger aliquots because we expected comparatively low ^{14}C activities in these samples. The ^{14}C counting efficiency of the scintillation spectrometer (LS 6500 Multi-Purpose Scintillation Counter, Beckman, USA) was at least 90% and the measurement error did not exceed 4%. The absolute ^{14}C activity was standardized by the H number method, using a ^{137}Cs external standard.

^{14}C activity of the washing water from the rhizosphere and non-rhizosphere loess was measured on 4 ml aliquots for total dissolved carbon and for DOC after release of CO_2 from DIC by adding 0.2 ml of 1 M HCl. ^{14}C activity of DIC was calculated by subtracting the ^{14}C in washing water before and after DIC release.

To measure ^{14}C incorporated into loess carbonate, we did not combust the loess samples (the usual method for soil samples) but rather dissolved the samples by acid, as combustion would lead to CO_2 release not only from carbonate but also from organic matter accumulated in loess by plant growth. Two grams of every dried loess sample (rhizosphere and non-

rhizosphere) were treated with 15 ml of 3 M H_3PO_4, and the CO_2 evolving from $CaCO_3$ was trapped in 12 ml of 2 M NaOH during 4 h to assure complete CO_2 absorption. ^{14}C activity of loess carbonate was measured on 6 ml aliquots of NaOH added to 12 ml of scintillation cocktail. Prior to ^{14}C analyses in loess carbonate, this method was tested for reliability by treating a sample of strongly ^{14}C enriched ryegrass shoot biomass with 3 M H_3PO_4 in the same way as described above. Subsequent ^{14}C analyses revealed a lack of ^{14}C in NaOH, showing that no organic C was released from shoot biomass by phosphoric acid.

Total inorganic and organic carbon contents in washing water were measured using a N/C analyzer ("Multi N/C 2100", AnalytikJena, Germany). Total carbon content of respired CO_2 trapped in NaOH was measured by titrating 0.1 ml aliquots with 0.01 M HCl against phenolphthalein after adding 1 M $BaCl_2$ solution (Zibilske 1994).

2.5 Calculations of carbonate recrystallization rate and statistical analysis

The ^{14}C results are presented as percentages of total assimilated ^{14}C ($^{14}C_{ass}$), which was calculated by subtracting the ^{14}C activities of the label residue ($^{14}C_{res}$) and of unassimilated CO_2 trapped in NaOH ($^{14}C_{NaOH}$) from the input ^{14}C activity ($^{14}C_{input}$).

$$^{14}C_{ass} = {}^{14}C_{input} - {}^{14}C_{res} - {}^{14}C_{NaOH} \quad (1)$$

The ^{14}C specific activity ($^{14}C_{SA}^{CO2}$) of CO_2 respired by roots and rhizomicrobial microorganisms was calculated as the ratio of ^{14}C activity ($^{14}C^{CO2}$) and total C content in respired CO_2 (C_t^{CO2}):

$$^{14}C_{SA}^{CO2} = \frac{^{14}C^{CO2}}{C_t^{CO2}} \quad (2)$$

We assume that the ^{14}C specific activities of respired CO_2 and of C that was incorporated into the loess carbonate by recrystallization and formation of secondary $CaCO_3$ are equal. Therefore, the amount of recrystallized $CaCO_3$ ($C_{t_{recryst}}^{CaCO3}$) was calculated according to equation 3, based on ^{14}C activity of loess $CaCO_3$ ($^{14}C^{CaCO3}$). The open pots were not sealed to allow an undisturbed exchange of air inside and outside of the pots. Therefore, the carbonate recrystallization in these pots better reflect the natural conditions. However, the ^{14}C specific activity in the pots was diluted by atmospheric CO_2. Thus, to calculate the amount of recrystallized $CaCO_3$ (Eq. 3), we used the average ^{14}C specific activity of respired CO_2 in sealed pots with the respective number of applied isotopic pulses, as the CO_2 respired by roots and rhizosphere microorganisms is identical in open and closed pots:

$$C_{t_{recryst}}^{CaCO3} = \frac{^{14}C^{CaCO3}}{^{14}C_{SA}^{CO2}} \quad (3)$$

Finally, the recrystallization rate of the secondary $CaCO_3$ was calculated as the amount of recrystallized $CaCO_3$-C divided by the amount of total C content of the loess carbonate

(C_t^{CaCO3}) and by the time (t) between the first labeling and the respective sampling (25 days at maximum).

$$CaCO_3 \text{ recrystallization rate} = \frac{C_{t_{recryst}}^{CaCO3}}{C_t^{CaCO3} \bullet t} \quad (4)$$

Standard errors of means are presented in figures. Significance of differences between the treatments was analyzed by one-way ANOVA with $\alpha = 5\%$ significance level.

3 Results

3.1 Budget of assimilated ^{14}C

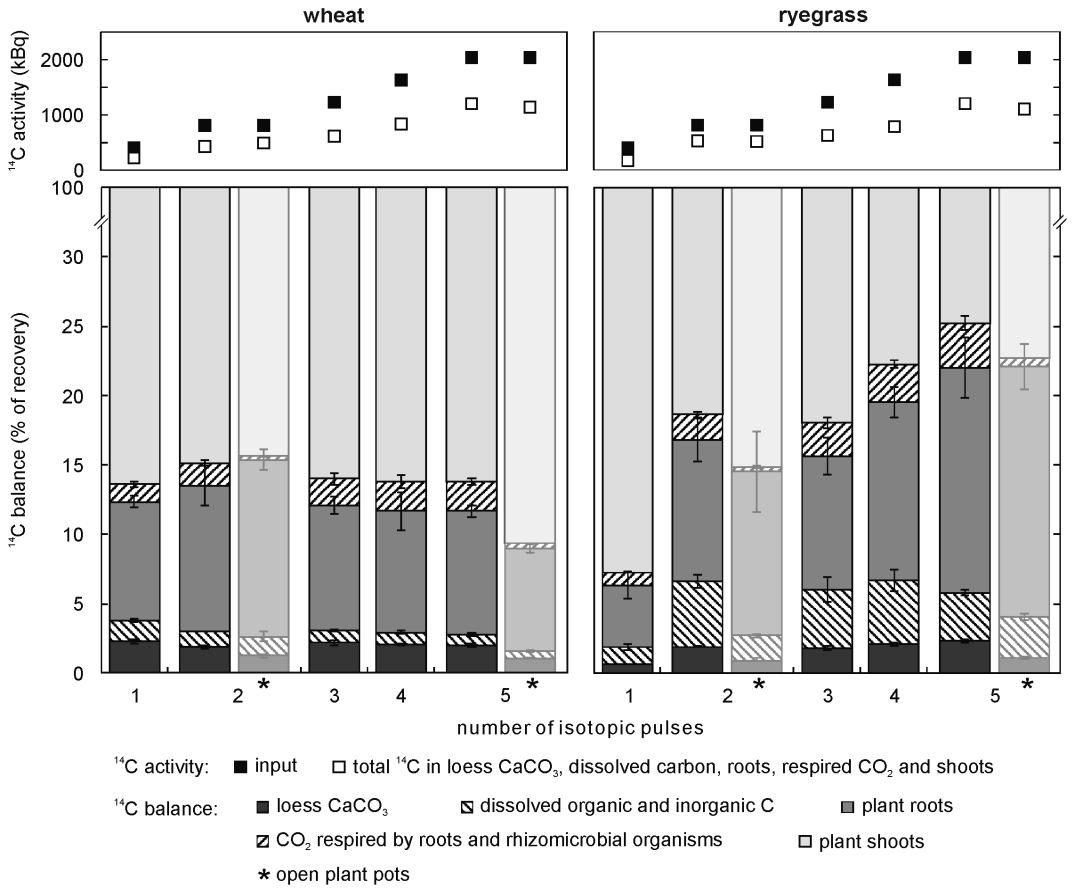

Fig. 3-1. Overview of ^{14}C dynamics for wheat and ryegrass. The upper diagrams show the totally recovered ^{14}C activities compared to the added ^{14}C label (summed up). A loss of up to 40% of the ^{14}C label by shoot respiration agrees with literature data (Kuzyakov et al. 2001). The lower diagrams show the distribution of recovered ^{14}C between above- and belowground C pools. Shaded columns represent the distribution patterns of unsealed plant pots.

Throughout the experiment, the largest percentage of assimilated ^{14}C was allocated in plant biomass. ^{14}C activities in shoots and roots of wheat plants remained approximately constant during the experiment, with shoot values fluctuating between 84.9 and 86.4% of recovered ^{14}C, and root values between 8.6 and 10.6% (Fig. 3-1). In contrast, ryegrass plants showed a distinct decrease of ^{14}C activity in shoots from 92.7 to 74.8% and an increase in roots from 4.5 to 16.2%. Accordingly, the total living plant biomass comprised up to 97% of

recovered ^{14}C. These values do not include the treatments with plant pots open for air exchange (shaded columns in Fig. 3-1). Due to $^{14}CO_2$ gas losses, the distribution patterns of recovered ^{14}C in these treatments are not in-line with the temporal changes observed in those treatments of sealed pots.

^{14}C activities in respired CO_2 reflect accumulated ^{14}C from the 1st pulse label to the respective sampling date. The part of assimilated ^{14}C found in respired CO_2 increased from 1.3 to 2.1% for wheat, and from 0.9 to 3.2% for ryegrass. In open plant pots these values remained low (0.7% at maximum) and did not increase as much as in sealed pots due to CO_2 and $^{14}CO_2$ losses to atmospheric air.

The most obvious differences between both plant species, concerning the balance of incorporated ^{14}C, were evident in loess carbonate and in dissolved C. In wheat, ^{14}C activity in loess carbonate varied slightly between 1.8 and 2.2%, and ^{14}C activity in dissolved C decreased from 1.5 to 0.8% from 1st to last sampling date. In ryegrass, the corresponding values were an increase from 0.7 to 2.3% and an increase from 1.2% to values between 3.5 and 4.7%.

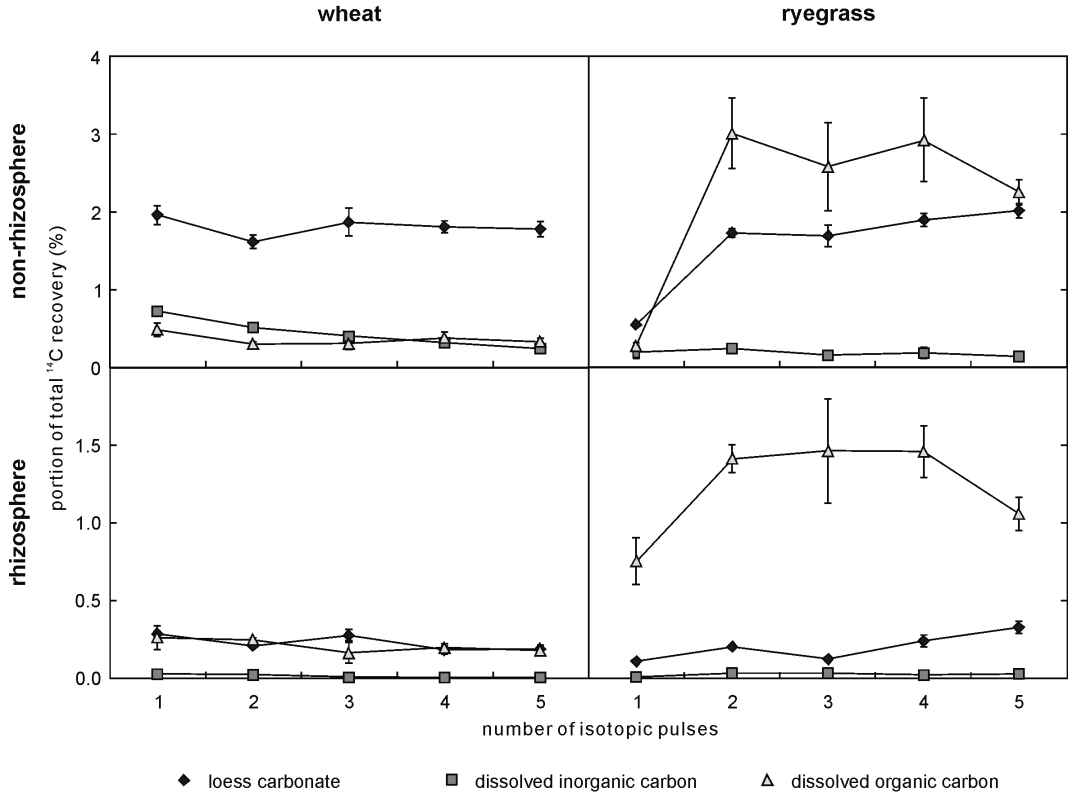

Fig. 3-2. ^{14}C activities recovered in loess carbonate, DIC and DOC of non-rhizosphere and rhizosphere loess as percentage of total ^{14}C recovery.

A closer look at ^{14}C recoveries in loess carbonate, DIC and DOC from rhizosphere and non-rhizosphere substrate shows that, for wheat, the recovery in both non-rhizosphere and rhizosphere loess $CaCO_3$ remained approximately constant (Fig. 3-2). The latter is approximately 10 - 14% of total ^{14}C activity recovered in loess carbonate. In non-rhizosphere loess, the percentage of recovered ^{14}C activity in DIC was initially higher than in DOC, decreased permanently, and was lower than DOC from the 4th sampling on. In rhizosphere

loess, the percentage of DIC-^{14}C was much lower than DOC-^{14}C and decreased down to zero whereas DOC remained constant (Fig. 3-2, left).

In ryegrass, ^{14}C recovery increased more consistently in non-rhizosphere versus rhizosphere loess carbonate, the latter containing between 6 and 16% of total loess carbonate ^{14}C activity. In non-rhizosphere loess, the percentage of ^{14}C recovered in DIC remained constant and was lower than DOC from the onset, while ^{14}C in DOC increased strongly between 1st and 2nd sampling. In rhizosphere loess, the percentage of ^{14}C in DIC remained low with little variation, while in DOC it first showed an increase, then slightly decreased (Fig. 3-2, right).

Dissolved inorganic carbon (DIC) consists of CO_2(aq), HCO_3^- and CO_3^{2-}. Dissolved organic carbon (DOC) comprises root exudates and microbial decomposition products. The absolute recovery of ^{14}C activity increased in both of these C pools during the experiment in non-rhizosphere loess. However, the steeper incline of ^{14}C activity in DOC, leading to a decreasing $^{14}C_{DIC}/^{14}C_{DOC}$ ratio, reflects increasing amounts of root exudates remaining in the loess during plant growth. At the same time, the ^{14}C in inorganic carbon in solution (DIC) cannot be accumulated because it undergoes recrystallization with and incorporation into $CaCO_3$. In loess adjacent to roots (rhizosphere loess), equal (wheat) or even higher (ryegrass) percentages of ^{14}C were recovered in DOC versus loess $CaCO_3$ due to root exudation.

During plant growth, assimilated ^{14}C was incorporated also in solid organic compounds in loess (e.g. lipids), released either by plant roots or by rhizomicrobial organisms decomposing plant remains. The above-described ^{14}C distribution patterns neglect this organic C pool in loess. Lipid extraction from ryegrass loess samples showed that in non-rhizosphere and rhizosphere loess, extractable lipids contribute merely 0.08 - 0.15% and 0.01 - 0.02% to the totally recovered ^{14}C, respectively (Gocke et al., unpublished data).

3.2 Calculated recrystallization rates in rhizosphere and root-free loess

The amount of recrystallized loess $CaCO_3$ as a portion of total loess carbonate was calculated for each of the five sampling dates in sealed and in open plant pots. The calculation is based on ^{14}C activity in loess $CaCO_3$ and ^{14}C specific activity of CO_2 evolved by root and rhizomicrobial respiration. As ^{14}C specific activities of $^{14}CO_2$ in open pots was low due to losses to atmospheric air, the amounts of recrystallized carbonate in open pots were calculated using the average ^{14}C specific activities of $^{14}CO_2$ in sealed pots from the same sampling date, and the ^{14}C activity recovered in loess.

Although the percentage of ^{14}C recovered in loess carbonate did not change significantly during the experiment (Fig. 3-1; for ryegrass from 2nd sampling on), calculated amounts of recrystallized carbonate increased from the first to the last sampling date (Fig. 3-3). The absolute ^{14}C activity in loess $CaCO_3$ as well as the total ^{14}C recovery increased from 1st to 5th sampling due to addition of equal ^{14}C pulses at every labeling (see upper diagrams in fig. 3-1).

Wheat as well as ryegrass showed linearly increasing amounts of recrystallized carbonate up to day 25 of the labeling (Fig. 3-3). In the *non-rhizosphere loess* samples, the recrystallized

portion of loess $CaCO_3$ in sealed plant pots rose from 0.06 to 0.15% (wheat) and from 0.02 to 0.16% (ryegrass). As the graph of the trend curves intersected the time axis approximately at the date of the 1st pulse label, we set the intersection at zero and recalculated the slopes. The slopes then corresponded to recrystallization rates of $6.2 \cdot 10^{-5}$ day^{-1} (wheat) and $6.1 \cdot 10^{-5}$ day^{-1} (ryegrass). At average, the recrystallization in open plant pots was approximately half as fast as in sealed pots despite similar ^{14}C distribution patterns in both treatments (see Fig. 3-1). The CO_2 concentration in the open plant pots reflected more likely the natural conditions in soil. For this reason, the slope of the recrystallization lines for the open plant pots (Fig. 3-3) expresses the actual $CaCO_3$ recrystallization rates under laboratory conditions of $3.2 \cdot 10^{-5}$ day^{-1} for wheat and $2.8 \cdot 10^{-5}$ day^{-1} for ryegrass (Tab. 3-1).

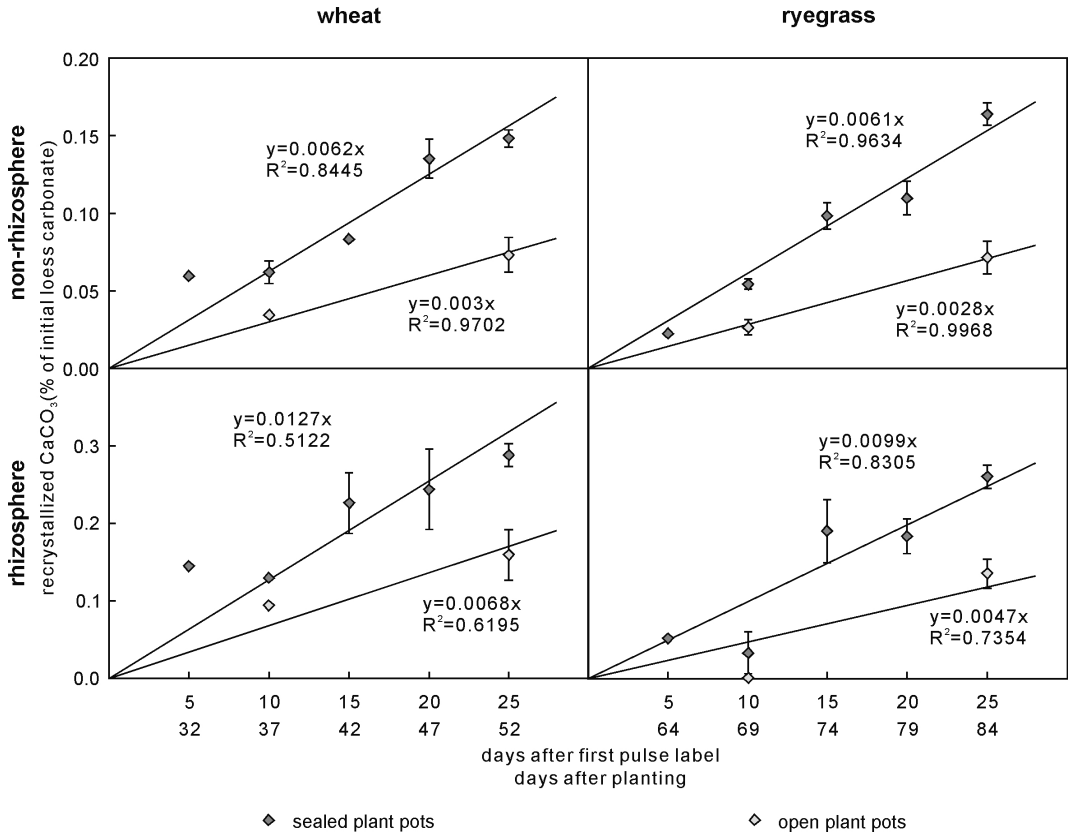

Fig. 3-3. Percentage of loess $CaCO_3$ recrystallized since the first pulse label in "root-free" loess samples (non-rhizosphere loess) and in loess adjacent to roots (rhizosphere loess) for wheat (left) and ryegrass (right). The linear trends reflect the recrystallization rates as % of initial $CaCO_3$ day^{-1}.

Table 3-1
Recrystallization rates calculated based on ^{14}C incorporated into loess $CaCO_3$ in different treatments.

Recrystallization rates (day^{-1})	Wheat		Ryegrass	
	Root-free	Rhizosphere	Root-free	Rhizosphere
Sealed pots	$6.2 \cdot 10^{-5}$	$12.7 \cdot 10^{-5}$	$6.1 \cdot 10^{-5}$	$9.9 \cdot 10^{-5}$
Open pots	$3.2 \cdot 10^{-5}$	$6.8 \cdot 10^{-5}$	$2.8 \cdot 10^{-5}$	$4.7 \cdot 10^{-5}$

The sampled *rhizosphere loess* comprised only 6 mass-% of the total amount of loess (average of all sampling dates). However, 12.5% of ^{14}C recovered in loess carbonate was

allocated in the rhizosphere. This suggested higher recrystallization intensity near plant roots, which was confirmed by the calculated amounts of recrystallized $CaCO_3$: At the last sampling date, recrystallized portions of loess carbonate in open plant pots with wheat were 0.07% and 0.16% in root-free and rhizosphere loess, reflecting recrystallizaton rates of $3.2 \cdot 10^{-5}$ day^{-1} and $6.8 \cdot 10^{-5}$ day^{-1}, respectively (Fig. 3-3). In contrast to non-rhizosphere loess samples, the calculated amounts in rhizosphere loess show rather high standard errors of the mean and, in the case of ryegrass, a rather high deviation from the linear trend (Fig. 3-3). The reason is a sampling-related irregular distinction between rhizosphere and non-rhizosphere material, leading to different portions of non-rhizosphere material in the rhizosphere samples. Nevertheless, in the case of wheat, the rates in the rhizosphere and root-free loess differed significantly from each other.

4 Discussion

4.1 Estimation of $CaCO_3$ recrystallization rates using the ^{14}C isotopic exchange approach

Based on the exchange of primary loess $CaCO_3$-C with C from root and rhizomicrobial respiration, we applied the isotopic exchange approach with ^{14}C as tracer to estimate the recrystallization rate of loess $CaCO_3$. Despite removal of root and rhizomicrobial respired CO_2 prior to each subsequent labeling, an artificial effect of the method on carbonate recrystallization can be excluded for the following reasons. First, we assume that CO_2 concentrations equal to the situation before CO_2 removal were reached within few hours because of permanent CO_2 supply over the time period of the experiment. Second, even if the removal of CO_2 prior to each labeling has entailed artificial precipitation of secondary carbonate, this presumably small portion of the total amount of secondary carbonate formed with ^{12}C and ^{13}C instead of ^{14}C and therefore was not included in the calculations based on ^{14}C activities in secondary carbonate. Therefore, the ^{14}C isotopic exchange approach yields reliable results and does not lead to artificially high recrystallization rates despite CO_2 removal from loess air prior to each labeling.

The method was first used to estimate pedogenic carbonate recrystallization by Kuzyakov et al. (2006). Their results as well as our current study show that the methodological sensitivity is high enough to detect the very low $CaCO_3$ recrystallization rates. Both studies yielded similar initial recrystallization rates of about $3 \cdot 10^{-5}$ day^{-1} in loess not adjacent to roots (non-rhizosphere loess), although growing conditions (nutrient supply) and experimental layout (plant species and age at the day of the first isotopic pulse) were not identical. This shows the good reproducibility of the ^{14}C isotopic exchange approach. Despite the very slow process, we were able to reproduce the former estimate, even with a modified experimental design. Nonetheless, our understanding of carbonate recrystallization in soils remains limited by the artificial conditions of the experiment. Especially the restricted space for root growth hinders direct extrapolation to field conditions.

4.2 Influence of plant species on ^{14}C dynamics and $CaCO_3$ recrystallization

We chose two plant species: 1) wheat (*Triticum aestivum*) to represent a typical agricultural cereal, and 2) ryegrass (*Lolium perenne*) to represent pasture grasses. The stage of plant development at the date of the first ^{14}C pulse differed for these species because ryegrass grows much slower than wheat.

The root biomass of wheat remained constant during the experiment, while root amounts in ryegrass increased from the first to the last sampling date. It is common for perennial plants that they increase C allocation belowground during the first year. Similar patterns were shown by the ^{14}C distribution: constant or even decreasing percentage of assimilated ^{14}C were recovered in belowground C pools of wheat, while strongly increasing percentages of ^{14}C were found in belowground C pools of ryegrass (Fig. 3-1, 3-4).

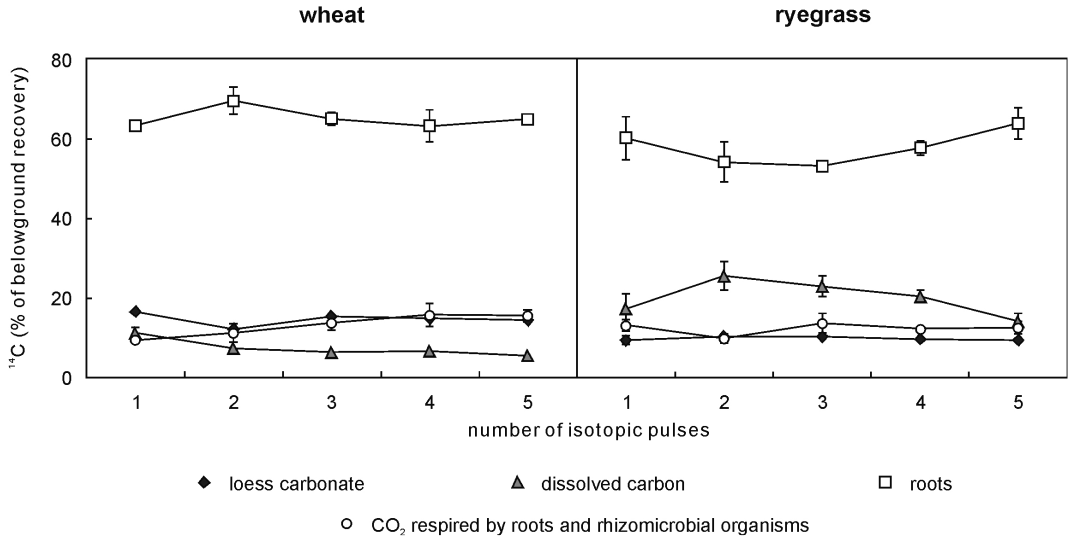

Fig. 3-4. Distribution of recovered ^{14}C between belowground C pools as percentage of total belowground ^{14}C activity.

Throughout the experiment and for both species, shoots comprised a much higher percentage of incorporated ^{14}C than roots. The distribution of total labeled and unlabeled C in plant biomass cannot be concluded directly from the distribution of the ^{14}C label (Kuzyakov & Domanski 2000). However, the recorded temporal changes in ^{14}C distribution patterns (Fig. 3-1) roughly represent the natural situation: during plant growth, the percentage of belowground translocated assimilated C decreases for cereals (e.g. Palta & Gregory 1998), while it increases for pasture grasses (Kuzyakov et al. 2001). The increasing part of ^{14}C recovered in ryegrass roots (from 4.5 to 16.2%) demonstrates the ascending C allocation to belowground pools by the pasture grass.

Despite different ^{14}C distribution patterns, both species showed similar recrystallization rates of approximately $3 \cdot 10^{-5}$ day^{-1} for non-rhizosphere loess. The replicates showed no significant difference between the rate in wheat and that in ryegrass. This was surprising because we expected different rooting properties of agricultural and pasture plants to result in differing carbonate recrystallization rates: In our study, more belowground plant biomass and thinner roots of ryegrass versus wheat provided more root surface for rhizosphere processes

(respiration, exudation). This situation is reflected by higher percentages of recovered ^{14}C in ryegrass dissolved organic carbon (DOC) than in wheat DOC (Fig. 3-2). Several reports show that pasture plants translocate approximately 30 - 50% of assimilated C into the soil (e.g. Swinnen 1994), compared with only 20 - 30% for cereals (e.g. Meharg & Killham 1990, 1991) as a result of breeding. This discrepancy, also found in field studies (Mensah et al. 2003), may be compensated by higher total CO_2 assimilation of cereals because of higher productivity (Kuzyakov & Domanski 2000), resulting in nearly equal C input of pasture and agricultural plants (1,500 kg C ha^{-1} $year^{-1}$) if the same time periods of growth are considered (Whipps 1990, Martin & Merckx 1992, van Ginkel et al. 1997).

In a study without living plants, the recrystallization rate strongly depended on the CO_2 concentration in soil air, with the graph describing a saturation curve where maximum amounts of recrystallized $CaCO_3$ were obtained at a CO_2 concentration of 5% (Gocke et al. 2010). For wheat and ryegrass, the CO_2 concentration in soil air of open plant pots averaged 6.7 ± 0.5% and 6.3 ± 0.5%. This probably explains the similar recrystallization rates.

Greater differences in plant characteristics (e.g. part of assimilated C used for belowground processes, respiration rates, exudation rates, growth rates, amount of fine roots, etc.) could lead to more widely diverging rates.

4.3 Effect of root vicinity on recrystallization rates

The carbonate recrystallization rates calculated in loess adjacent to roots were approximately twice as high as in “root-free” loess (Tab. 3-1). This shows the importance of vegetation for the rates of secondary $CaCO_3$ formation. The three main factors controlling dissolution and precipitation of $CaCO_3$ are: (1) CO_2 partial pressure in pore space, (2) pH of soil solution, and (3) mass flow of dissolved carbon species.

According to the Henry Law, the solubility of CO_2 increases directly proportional to CO_2 partial pressure. This means that high CO_2 concentration in soil air should lead to a higher concentration of H^+ in soil solution by dissociation of carbonic acid, and thus to a decreasing pH. High $CaCO_3$ content in loess buffers these pH variations. Nevertheless, the dependence of $CaCO_3$ recrystallization on CO_2 concentration is evident when comparing sealed and open plant pots: In sealed pots, both CO_2 concentrations (11.1% for wheat, 17.7% for ryegrass) and recrystallization rates were twice or three times as high as in open pots allowing air exchange with the atmosphere. Independent of plant species, accumulation of respired CO_2 leads to enhanced dissolution and recrystallization of primary $CaCO_3$. This is supported by a previous study without living plants (Gocke et al. 2010) which showed that the recrystallization intensity increases with ascending CO_2 concentration in a range between 380 and 50000 ppm CO_2. Under field conditions, soil CO_2 concentrations typically vary between the atmospheric value (around 0.04%) and 100 times the atmospheric value (Davidson 1995). However, only few studies distinguish between CO_2 concentrations in the rhizosphere and those in root-free soil. Gollany et al. (1993) showed that pCO_2 decreases smoothly with increasing distance to the root surface of sorghum plants within the first few millimeters. Hinsinger et al. (2003) suggest that the effect of CO_2 released by root and rhizomicrobial respiration is locally limited

because of the higher diffusivity of CO_2 when compared to dissolved inorganic C species. That means that high CO_2 concentration occurs only locally, adjacent to the plant roots where CO_2 supply is constant. Our present data confirm this: recrystallization rates were significantly higher in the rhizosphere versus "root-free" loess of wheat and ryegrass.

The pH in soil and especially in rhizosphere of living plants is affected by several interacting factors. These were listed by Hinsinger et al. (2003) as (1) bulk soil pH and pH buffering capacity, (2) H^+ or OH^- release by roots for a neutral anion/cation balance, (3) release of organic anions from fatty acids, (4) root exudation and respiration, (5) redox-coupled processes, (6) environmental and nutritional factors. Moreover, as revealed by Gras (1974) and Nye (1981), the effect of increased root and rhizomicrobial respiration on rhizosphere pH becomes much more noticeable in calcareous than in acidic soils. Some of these factors were affected in our study by using a nutrient solution that contained nitrogen (N) solely as NO_3^-, resulting in OH^- release by plant roots, and by applying loess as a growth medium (pH (H_2O) ≈ 8.4, $CaCO_3$ content 29%).

CO_2 release by roots and rhizosphere microorganisms is at least 1 order of magnitude higher compared to H^+ or OH^- release by roots (Lambers et al. 1996, Durand & Bellon 1993, Durand et al. 2001). Thus, high CO_2 concentrations (also in the open plant pots) were one of the main factors promoting and accelerating loess $CaCO_3$ recrystallization in general, and especially in the rhizosphere. In arable soils, however, the rhizosphere pH and $CaCO_3$ recrystallization intensity could also be affected by fertilizer composition (type of added N as anions or cations, amount of PO_4^{3-}). Yongliang et al. (2001) measured a remarkable decrease of rhizosphere pH compared with bulk soil after applying NH_4^+ fertilizer to pines. Moreover, soil fertility management can increase the recrystallization rate by increasing the belowground biomass, promoting CO_2 flux (Lal & Kimble 2000).

Our sampling design did not permit determining the extent of the rhizosphere effect on loess carbonate recrystallization as a function of distance to the root surface. We suggest, however, that considerably higher rates are limited to the first few millimeters adjacent to the roots. A more detailed view on this relation is desirable and might be possible by applying special sampling approaches: e.g. platy plant boxes (rhizotrons), or separation of soil layers with increasing proximity to the root surface (Kuzyakov et al. 2003). Regarding longer time scales than in our study (years to centuries), the effects of CO_2 partial pressure and rhizosphere pH might be minor compared with the effect of water movement and soil moisture changes. Transport of dissolved Ca^{2+} and HCO_3^- towards the root might enhance $CaCO_3$ precipitation in a diameter of up to several centimeters around the roots. This is possible in calcareous sediments, where high amounts of Ca^{2+} in the rhizosphere can lead to the formation of encrustations around roots termed rhizoliths (Klappa 1980). A high $CaCO_3$ content together with homogeneity and high porosity in loess or calcareous sands (e.g. dune sands) as well as active, long-living roots provide a high potential for such rhizolith formation (Gocke et al. unpublished data).

4.4 Extrapolation of $CaCO_3$ recrystallization rates over longer periods

Two approaches for extrapolating the initial recrystallization rate over longer time periods can be considered. In the first approach, the precondition is that every molecule of primary $CaCO_3$ is affected by recrystallization once, and thereafter removed from recrystallization, e.g. by formation of concretions. This corresponds to a linear decrease of the remaining primary $CaCO_3$ with the slope of obtained recrystallization rates. Such a linear decrease translates into complete recrystallization after 100 years at maximum (Fig. 3-5 a, b). The second approach assumes repeated recrystallization of the calcium carbonate. This assumption results in an exponential decrease of remaining primary carbonate with recrystallization periods between several hundreds of years and more than 1000 years, depending on the length of the growing season (see Kuzyakov et al. 2006). The amount of recrystallized carbonate ($CaCO_3(t)$) is calculated as follows:

$$CaCO_3(t) = 100 \bullet (1 - e^{-t \bullet rate \bullet \frac{GS}{365}}) \quad (5)$$

with t: time in days, rate: recrystallization rate in day^{-1}, GS: growing season in days. In the following, if the exponential approach is considered, the term recrystallization period means the time necessary for recrystallization of 95% of the $CaCO_3$ initially present in loess. A remaining percentage of 5% of primary carbonate is too small to be detected by mass spectrometric analysis of the natural abundances of stable C isotopes ($\delta^{13}C$).

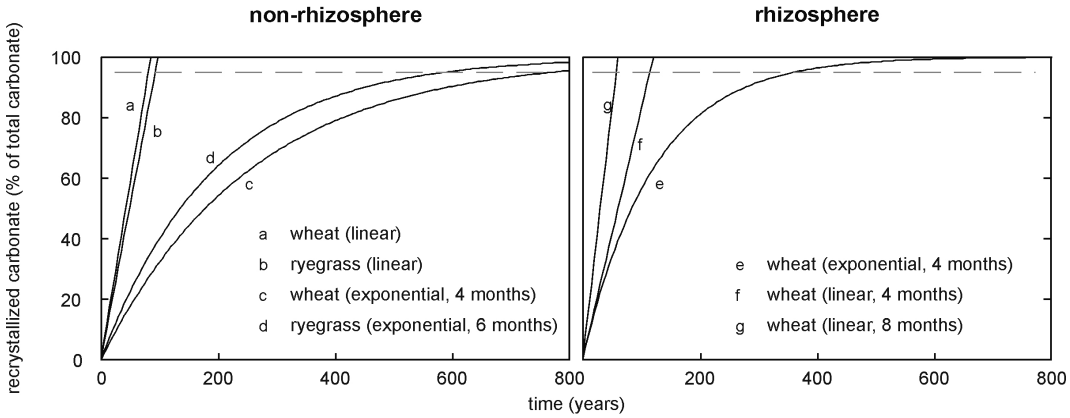

Fig. 3-5. Recrystallization periods extrapolated by linear and exponential models as depending on the root vicinity and growth season.

We modeled the recrystallization periods with typical growing seasons, i.e. 4 months for wheat and 6 months for ryegrass, and calculated the periods necessary for the recrystallization of 95% of primary loess carbonate (Fig. 3-5 c, d). The result was about 800 years for wheat and 600 years for ryegrass. In contrast, only 360 years would be necessary for 95% recrystallization of $CaCO_3$ in rhizosphere of wheat (Fig. 3-5 e). Clearly, the formation of secondary carbonate in soil may be faster directly on the root surface. An extreme case of such a root-mediated process is rhizoliths (Klappa 1980, Gocke et al. unpublished data) and calcified root cells (Pustovoytov & Terhorst 2004, Wang & Greenberg 2007). After the formation of initial secondary $CaCO_3$ portions, the root may die. The secondary carbonate crystals, however, may grow further because they have much smaller volume to mass ratio compared to dispersed (primary or secondary) $CaCO_3$ in loess. In case of rhizolith formation,

secondary carbonate is removed immediately and will not be affected by recrystallization again. Recrystallization should thus lead to a linear decrease of the amount of remaining primary carbonate. Applying the linear approach to the rate of wheat (growing season 4 months), we extrapolated a recrystallization period of 120 years for recrystallization of 95% of primary $CaCO_3$ (Fig. 3-5 f). However, due to a relatively low nutrient content of loess, plants growing on this sediment are predominantly evergreen plants with growing seasons of up to 8 months. This would potentially lead to a complete (95%) recrystallization within about 60 years (Fig. 3-5 g). These periods are just a rough approximation because they were extrapolated with rhizosphere recrystallization rates of wheat. Rhizoliths, however, more likely were formed by bush or tree vegetation (Gocke et al. unpublished data), whose initial recrystallization rates are unknown. Nevertheless, our modeled data agree with the assumption that rhizoliths form rather fast (decades or even years) during the plant's life time and/or beginning decay (e.g. Wang & Greenberg 2007, Klappa 1980).

As mentioned above, the chronological resolution of paleoenvironmental studies based on pedogenic carbonates is limited not by the analytical precision of the instruments (IRMS for $\delta^{13}C$ or AMS for $\Delta^{14}C$), but by the period necessary for secondary carbonate formation. This should be considered when carbonates are used for high-resolution paleoclimatic and paleoecological studies (e.g. Wang & Greenberg 2007).

5 Conclusions

The ^{14}C isotopic exchange approach is a very sensitive and reliable tool for estimating low $CaCO_3$ recrystallization rates. It is currently the only approach to estimate recrystallization rates of carbonates in soil based on isotopic exchange with $^{14}CO_2$ released in the rhizosphere after plant labeling. Using this approach, we calculated a very low initial loess $CaCO_3$ recrystallization rate of $3 \cdot 10^{-5}$ day^{-1}, which is in accordance with literature data. Similar rates were calculated in root-free loess from wheat and ryegrass, but loess adjacent to roots showed rates that were more than twice as high. This reflects the importance of root respiration and microbial respiration by decomposition of root exudates for $CaCO_3$ recrystallization and becomes relevant especially for exponential extrapolation of rates on recrystallization periods: In non-rhizosphere loess, primary $CaCO_3$ would be completely recrystallized after hundreds of years, depending on the plant's growing season, while the process would be considerably shorter within the rhizosphere. The relation between the carbonate recrystallization rate and the distance to roots is a very important aspect determining the process duration. Therefore, it merits further investigation.

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Study 4: Pedogenic carbonate recrystallization rates and periods are regulated by temperature-dependent rhizosphere processes: Relevance for paleoenvironmental applications

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Abstract

In soils of arid and semiarid climates, dissolution of primary (lithogenic) carbonate and recrystallization with CO_2 from soil air leads to precipitation of pedogenic carbonates and formation of calcic horizons. Thus, their carbon isotope composition represents the conditions prevailing during their formation. However, the widespread use of the isotopic signature ($\delta^{13}C$, $\delta^{18}O$, $\Delta^{14}C$) of pedogenic carbonates for reconstruction of local paleovegetation, paleoprecipitation and other environmental conditions lacks knowledge of the time frame of pedogenic carbonate formation, which depends on climatic factors. We hypothesized that temperature-dependent biotic processes like plant growth and root and rhizomicrobial respiration have stronger influence on soil $CaCO_3$ recrystallization than abiotic temperature-dependent solubility of CO_2 and $CaCO_3$.

To assess the effect of temperature on initial $CaCO_3$ recrystallization rates, loess with primary $CaCO_3$ was exposed to $^{14}CO_2$ from root and rhizomicrobial respiration of plants labeled in $^{14}CO_2$ atmosphere at 10, 20 or 30 °C. ^{14}C recovered in recrystallized $CaCO_3$ was quantified to calculate amounts of secondary $CaCO_3$ and corresponding recrystallization rates, which were in the range of 10^{-4} – 10^{-6} day^{-1}. Increasing rates with increasing temperature showed the major role of biological activities like enhanced water uptake by roots and respiration. The abiotic effect of lower solubility of CO_2 in water by increasing temperature was completely overcompensated by biotic processes. Based on initial recrystallization rates, periods necessary for complete recrystallization were estimated for different temperatures, presuming that $CaCO_3$ recrystallization in soil takes place mainly during the growing season. Taking into account the shortening effect of increasing temperature on the length of growing season, the contrast between low and high temperature was diminished, yielding recrystallization periods of 5740 years, 4330 years and 1060 years at 10, 20 and 30 °C, respectively. In summary, increasing $CaCO_3$ recrystallization rates with increasing temperature demonstrated the important role of vegetation for pedogenic $CaCO_3$ formation and the predominantly biotic effects of growing season temperature.

Considering the long periods of pedogenic carbonate formation lasting to some millennia, we conclude that methodological resolution of paleoenvironmental studies based on isotope composition of pedogenic carbonates is limited not by instrumental precision but by the time frame of pedogenic carbonate formation and hence cannot be better than thousands of years.

Keywords: pedogenic $CaCO_3$ / recrystallization periods / ^{14}C isotopic exchange / temperature / biotic effects / root and rhizomicrobial respiration

1 Introduction

Pedogenic (secondary) carbonate is a common constituent of continental sediments and soils as well as paleosols of arid to subhumid climates (Eswaran et al., 2000), mostly with mean annual precipitation of less than 500 mm (Birkeland, 1999). Pedogenic $CaCO_3$ forms by precipitation of Ca^{2+} from soil minerals (e.g. primary $CaCO_3$) or external sources with dissolved CO_3^{2-} (Borchardt and Lienkaemper, 1999). This precipitation is caused by a

decrease of CO_2 partial pressure, increase of Ca^{2+} and HCO_3^- concentrations, or a combination of both (Krauskopf and Bird, 1995; Birkeland, 1999).

The usually good preservation of pedogenic carbonates in arid and semiarid climates makes them an important tool for the assessment of paleoclimatic conditions and paleovegetation. The link between the photosynthetic pathway of vegetation (C_3 or C_4 plants) and the $^{13}C/^{12}C$ ratio in pedogenic carbonates was established when Cerling (1984) showed that carbon (C) in pedogenic carbonates does not derive from lithogenic $CaCO_3$ of soil parent material, but from soil CO_2. With the latter being released mainly by respiration of roots and associated microorganisms (Amundson et al., 1998), $\delta^{13}C$ analyses of pedogenic carbonates has become a widespread method to determine the assemblage of the predominant local paleovegetation (e.g. Quade and Cerling, 1995; Buck and Monger, 1999; Ding and Yang, 2000; Kovda et al., 2006; Pustovoytov et al., 2007a). Additionally, $\delta^{18}O$ of pedogenic carbonates records paleotemperatures and –precipitation (Dworkin et al., 2005) because the former is related to the isotopic composition of meteoric water (Cerling, 1984). Recent studies have demonstrated the suitability of pedogenic carbonates for the clumped isotope approach (Ghosh et al., 2006), thereby enabling their use as paleothermometer. Furthermore, pedogenic carbonates are used to estimate the age of pedogenesis or of sediments by radiocarbon dating (e.g. Amundson et al., 1994; Pustovoytov et al., 2007b).

One important aspect of these paleoenvironmental and chronological studies is their temporal resolution, which is limited by the time frame of pedogenic carbonate formation (Royer et al., 2001) and of recrystallization by postsegregational alteration (Kuzyakov et al., 2006; Gocke et al., 2010a, b). Based on carbon isotope composition of soil carbonates ($\delta^{13}C$, Nordt et al., 1998; $\Delta^{14}C$, Pendall et al., 1994) within chronosequences of known age, formation periods of pedogenic carbonates were suggested to be in the range of thousands of years. However, these approaches face the problems of (1) too low sensitivity of ^{13}C natural abundance for differentiation of small amounts of C involved in isotopic exchange between carbonate and soil CO_2, and (2) often too low chronological resolution of chronosequences. In addition, due to very low rates of pedogenic carbonate formation and recrystallization, values obtained from field conditions usually represent average rates over large time periods during which unstable climatic conditions presumably led to considerably varying $CaCO_3$ formation and accumulation rates (Gile et al., 1981; McFadden and Tinsley, 1985). Therefore, initial rates of pedogenic $CaCO_3$ formation and their dependence on environmental factors (e.g. primary $CaCO_3$ content of parent material, precipitation and temperature) remain unknown.

So far, initial $CaCO_3$ recrystallization rates can be assessed only under controlled conditions, as introduced by Kuzyakov et al. (2006). Their approach uses of the C isotopic exchange of primary (lithogenic) $CaCO_3$ with ^{14}C-labeled CO_2 from soil air during recrystallization and formation of secondary $CaCO_3$. ^{14}C from rhizosphere respiration of plants labeled in $^{14}CO_2$ atmosphere in loess $CaCO_3$ was quantified to calculate amounts of secondary (recrystallized) $CaCO_3$. The authors calculated an initial recrystallization rate of $2.9 \cdot 10^{-5}$ day^{-1}. Extrapolation of this rate on longer time periods showed that at least 400 years, but probably thousands of years are necessary for complete recrystallization in loess

containing 27% $CaCO_3$. This approach was further applied to reveal the dependence of initial recrystallization rates on CO_2 concentration in loess pore space (Gocke et al., 2010a) as well as on root vicinity (Gocke et al., 2010b). These studies showed that $CaCO_3$ recrystallization rates strongly increase with increasing CO_2 concentration and therefore are higher in the rhizosphere than in loess distant from roots. Further environmental factors affect pedogenesis and might play an important role for the reactions between $CaCO_3$, CO_2 and H_2O.

Temperature is one of these factors. Pedogenic carbonates are abundant and were most often described in soils of warm climates, but were also observed in soils of colder climates, e.g. Alaska (Marion et al., 1991) and Spitzbergen (Courty et al., 1994). While the dependence of pedogenic carbonate accumulation rates and depth on amounts of rainfall has often been investigated (e.g. Arkley, 1963; McFadden and Tinsley, 1985; Marion, 1989; Nordt et al., 2006), the effect of temperature on initial $CaCO_3$ recrystallization rates was not quantified so far. Marion (1989) found no correlation between modern mean annual temperature and accumulation rates of Pleistocene calcic horizons. However, the role of temperature is reflected e.g. by its role in isotopic fractionation during secondary $CaCO_3$ formation (Romanek et al., 1992). Pustovoytov (2003) stated that increasing temperature can influence the intensity (i.e. thickness) of $CaCO_3$ accumulation on clasts in several ways which might partly counteract each other: via stimulation of evaporation, by controlling $CaCO_3$ solubility and by enhancing pCO_2 in soil.

Biotic processes like plant growth, root and rhizomicrobial respiration are promoted by increasing temperature which might lead to enhanced recrystallization at high temperature because of higher CO_2 concentration in soil. However, abiotic factors, mainly solubility of CO_2 and $CaCO_3$, decrease with increasing temperature. This controversy does not allow a simple prediction of the effects of temperature on formation of pedogenic carbonates and their recrystallization rates. Therefore, we evaluated these effects on initial $CaCO_3$ recrystallization rates and calculated periods experimentally by applying the ^{14}C isotopic exchange approach at different temperature levels. The aim of this work was to reveal, which temperature-controlled factor – abiotic effect of CO_2 and $CaCO_3$ solubility or intensity of biological activity – exerts more influence on secondary $CaCO_3$ formation and recrystallization in soils. Further aim was to confirm the assumption that faster recrystallization occurs in rhizosphere compared to loess distant from roots, as shown by Gocke et al. (2010b), and to reveal the influence of temperature on this effect.

2 Material and methods

2.1 Experimental layout and ^{14}C labeling

Maize (*Zea mays* [L.], cv. Tassilo) was chosen for the ^{14}C multiple pulse labeling experiment because of its fast growth and because this plant does not entail a strong decrease of pH in rhizosphere, which might lead to artificial promotion of $CaCO_3$ recrystallization. As shown before, in maize experiments a major part of soil CO_2 is derived directly from root biomass, compared to a small portion of microbial-derived CO_2 (Werth and Kuzyakov, 2008).

Plants were grown on loess from Nussloch, SW Germany with high $CaCO_3$ content (274 mg g^{-1}, Gocke et al., submitted) and low C_{org} content (0.3 mg g^{-1}, Wiesenberg et al., 2010). Loess was chosen to avoid interference of CO_2 fluxes by microbial decomposition of old organic matter, and to simulate initial pedogenesis on a calcareous sedimentary parent material which is globally abundant. Polycarbonate filtration devices (CombiSart, Sartorius AG, Germany) were used as plant pots, with three inlets in the lid and one main opening for growth of the plant shoots. In each plant pot with 420 g air-dry loess, one pre-germinated maize seedling was grown under controlled conditions of 14/10 h day/night periods, 20 °C and light intensity of 300 µmol m^{-2} s^{-1}, and loess moisture was adjusted to 70% of WHC (100% WHC = 28% of loess weight) once a day. Plants were kept in three plant growth chambers (Adaptis A1000, Conviron, Canada) during the entire experiment. For nutrient supply, plants were fertilized with Hoagland nutrient solution (Hoagland and Arnon, 1950) modified after Gocke et al. (2010b).

Two days before the first labeling, three treatments were separated by decreasing the temperature to 10 °C in one plant growth chamber and increasing it to 30 °C in another plant growth chamber. Thus, three levels of mean temperature during the growing season were simulated: 10, 20 and 30 °C. One day before the first ^{14}C labeling, plant pots were flushed with air to remove all previously respired CO_2, and three plants in each treatment were sealed around the shoots with silicone rubber (NG 3170, Thauer & Co., Germany) to prevent loss of respired CO_2 by air exchange between the loess-root compartment and the atmosphere.

Starting from an age of 14 days after planting, plants received 1 to 4 ^{14}C isotopic pulses á 407 kBq in intervals of 5 days. For this purpose, plants were exposed to artificially labeled $^{14}CO_2$ atmosphere in an airtight acrylic glass chamber for 3 hours. $^{14}CO_2$ was released by dissolving $Na_2{}^{14}CO_3$ (ARC Inc., USA) in H_2SO_4. A detailed description of the labeling procedure is given in Kuzyakov et al. (2006) and Gocke et al. (2010b). The experiment was conducted with 3 replications. Each temperature treatment also included time as a second factor, resulting in a total of 15 plant pots per treatment:

1) Plants in open pots, labeled once
2) Plants in open pots, labeled twice
3) Plants in open pots, labeled three times
4) Plants in open pots, labeled four times
5) Plants in sealed pots, labeled four times

In between the labelings, plants continued growth under normal conditions. In contrast to most other ^{14}C labeling studies, rhizosphere CO_2 was not flushed out of the loess-root compartment between the labeling procedures. This allowed accumulation of root-respired $^{14}CO_2$ and isotopic exchange between the latter and loess $CaCO_3$.

2.2 Sampling and ^{14}C analysis

Five days after each labeling, rhizosphere CO_2 accumulated in the loess-root compartments was flushed out and trapped in NaOH solution to form Na_2CO_3. An aliquot of

this solution was titrated with HCl against Phenolphthalein (Zibilske, 1994) to determine the amount of C in root-respired CO_2. Another aliquot was mixed with scintillation cocktail (Rotiszint, Roth, Germany) and analyzed for ^{14}C activity by a liquid scintillation counter (LS 6500 Multi-Purpose Scintillation Counter, Beckman, USA).

Afterwards, three plant pots were harvested for analyses (see below), while the remaining plants received the next ^{14}C isotopic pulse. From the plant pots selected for analyses, shoots were cut and roots were separated from loess with tweezers. This loess, in the following called non-rhizosphere loess, was washed with deionized water to remove dissolved organic and inorganic carbon (DOC, DIC). Rhizosphere loess, the material sticking to the roots, was obtained by washing roots with deionized water and filtration of this washing solution. All samples were dried at 90 °C and ground in a ball mill (MM200, Retsch, Germany).

To determine ^{14}C activity in loess $CaCO_3$, samples were treated with H_3PO_4 to release CO_2 only from loess $CaCO_3$ but not from organic compounds in loess (root fragments, exudates). CO_2 was trapped in NaOH and an aliquot of the solution was measured as described above.

Total organic and inorganic carbon contents in washing solution were measured using a N/C analyzer (Multi N/C 2100, AnalytikJena, Germany).

2.3 Calculation and statistics

Calculation of amounts of recrystallized $CaCO_3$ was based on ^{14}C incorporated in $CaCO_3$ by isotopic exchange with rhizosphere CO_2. We assume equal ratios of ^{14}C / total C for rhizosphere CO_2 and for C that was incorporated into the loess carbonate by recrystallization. Because ^{14}C was highly enriched compared to the level of natural abundance, the isotopic fractionation during the experiment was negligible. The ^{14}C / total C ratio (^{14}C specific activity, $^{14}C^{sp}_{CO_2}$) was calculated from accumulated CO_2 in sealed plant pots, where the original ratio was not altered by exchange with atmospheric air. Afterwards, amounts of recrystallized $CaCO_3$ ($CaCO_{3_{recryst}}$) were calculated based on carbonate ^{14}C activities in unsealed plant pots ($^{14}C_{CaCO_3}$), where CO_2 concentrations more likely reflected natural conditions (Eq. 1).

$$CaCO_{3_{recryst}} = \frac{^{14}C_{CaCO_3}}{^{14}C^{sp}_{CO_2}} \quad (1)$$

with $^{14}C^{sp}_{CO_2}$ as $^{14}C/Ct$ ratio of respired CO_2 in sealed plant pots and $^{14}C_{CaCO_3}$ as ^{14}C activity of loess $CaCO_3$ in unsealed plant pots.

The $CaCO_3$ recrystallization rate was determined according to Equation 2:

$$CaCO_3 \text{ recrystallization rate} = \frac{CaCO_{3_{recryst}}}{CaCO_{3_t} \bullet t} \quad (2)$$

with $CaCO_{3_t}$ as amount of total $CaCO_3$ and t as the time between first labeling and respective sampling.

Based on calculated rates, periods necessary for complete recrystallization of loess $CaCO_3$ were estimated (Eq. 3). Most likely, repeated recrystallization of primary and secondary $CaCO_3$ prior to definite precipitation of secondary $CaCO_3$ cause an exponential increase of secondary $CaCO_3$ (Kuzyakov et al., 2006). We considered the situation that 95% of total loess $CaCO_3$ was recrystallized, because under field conditions, a remaining portion of ≤ 5% of primary $CaCO_3$ is too small to be detected by isotope ratio mass spectrometry (IRMS) analysis on the level of ^{13}C natural abundance. Further, notable $CaCO_3$ recrystallization takes place predominantly during the growing season (Kuzyakov et al. 2006), when high soil CO_2 concentration and permanent CO_2 supply by root and rhizomicrobial respiration impede equilibrium of the $CaCO_3 - CO_2 - HCO_3^-$ system (Gocke et al., 2010a, b). Therefore, length of the growing season (GS) was taken into account in Equation 3:

$$t = \frac{-\ln(0.05)}{GS \bullet RR} \qquad (3)$$

with t as $CaCO_3$ recrystallization period, GS as growing season in days $year^{-1}$ and RR as recrystallization rate in $days^{-1}$.

Mean values and standard errors of the mean are presented in figures. The sample set was tested for significance of differences between temperature levels using one-way ANOVA with a significance level of $\alpha = 0.05$, followed by post hoc Scheffé test. Statistical analysis was carried out using STATISTICA for Windows (version 7.0, StatSoft Inc., Tulsa, USA).

3 Results

3.1 Amounts of recrystallized $CaCO_3$ and recrystallization rates

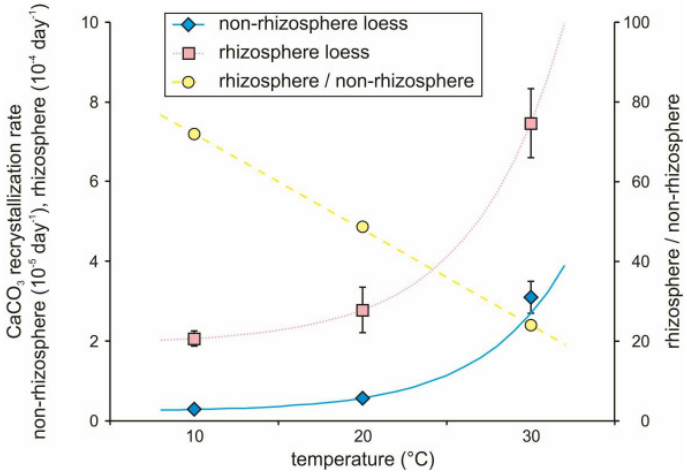

Fig. 4-1: Dependence of $CaCO_3$ recrystallization rates in non-rhizosphere and rhizosphere loess on temperature (note one order of magnitude different Y axes scales for rhizosphere and non-rhizosphere loess) and ratio of these rates in rhizosphere vs. non-rhizosphere loess.

From the first to the last sampling date, amounts of recrystallized $CaCO_3$ increased approximately in a linear way (not shown here). After four labeling procedures, i.e. 20 days

after the first labeling, portions of recrystallized $CaCO_3$ were at minimum $6.2 \cdot 10^{-3}$ % and at maximum 1.9% of total loess $CaCO_3$ (Tab. 4-1).

Considering the amounts of recrystallized $CaCO_3$ from all sampling dates, these portions corresponded to $CaCO_3$ recrystallization rates in the range of 10^{-6} – 10^{-4} day^{-1}. In each treatment, the rates were 20 to 70 times lower in non-rhizosphere loess compared to rhizosphere loess, and values were smallest for plants grown at 10 °C and highest for those grown at 30 °C (Fig. 4-1).

Within the range of applied temperatures (10 °C – 30 °C), the dependence of $CaCO_3$ recrystallization rates on temperature was described by an exponential increase in non-rhizosphere (Eq. 4) and rhizosphere loess (Eq. 5).

$$CaCO_3 \text{ recrystallization rate} = 1.3 \bullet 10^{-8} \bullet \exp(0.25 \bullet T) + 4.8 \bullet 10^{-6} \quad (4)$$

$$CaCO_3 \text{ recrystallization rate} = 9.3 \bullet 10^{-7} \bullet \exp(0.21 \bullet T) + 2.6 \bullet 10^{-4} \quad (5)$$

Table 4-1: Portions of $CaCO_3$ recrystallized in non-rhizosphere and rhizosphere loess 20 days after the first labeling.

amounts of recrystallized $CaCO_3$ [% of total $CaCO_3$]	10 °C	20 °C	30 °C
non-rhizosphere loess	0.0062 ± 0.0008	0.0112 ± 0.0025	0.0908 ± 0.0050
rhizosphere loess	0.3578 ± 0.0292	0.4876 ± 0.0674	1.8976 ± 0.1046

3.2 Respired CO_2 and dissolved inorganic and organic carbon

CO_2 concentration in the loess-root compartment after five days of accumulation did not change significantly between the four sampling dates within each treatment. Average CO_2 concentration in the unsealed pots was 2.2 ± 0.3 v-%, 5.8 ± 0.6 v-% and 6.0 ± 0.5 v-% at 10, 20 and 30 °C, respectively (Fig. 4-2).

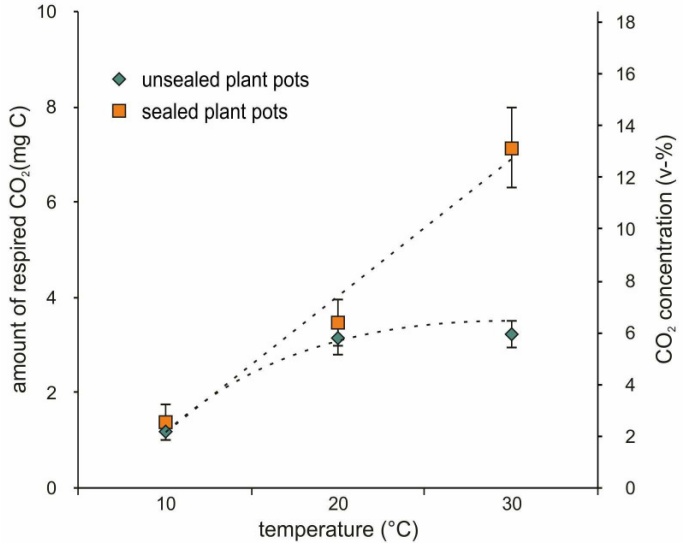

Fig. 4-2: Comparison of CO_2 concentrations at three temperatures in open and sealed plant pots. CO_2 concentrations represent the amount of CO_2 accumulated during 5 days.

Similar to CO_2 concentrations, amounts of dissolved inorganic carbon (DIC), which were not precipitated as secondary $CaCO_3$, did not change significantly with time for each treatment. After 5 weeks of plant growth in total, DIC contents of non-rhizosphere loess were only slightly increased compared to unplanted loess, and dissolved organic carbon (DOC) contents in unplanted and non-rhizosphere loess were within the same range (Fig. 4-3a, b). While DIC contents were significantly higher in rhizosphere loess (0.03 – 0.16 mg g^{-1} loess) compared to non-rhizosphere loess (0.02 – 0.06 mg g^{-1} loess), temperature had no effect on DIC contents (Fig. 4-3b, c).

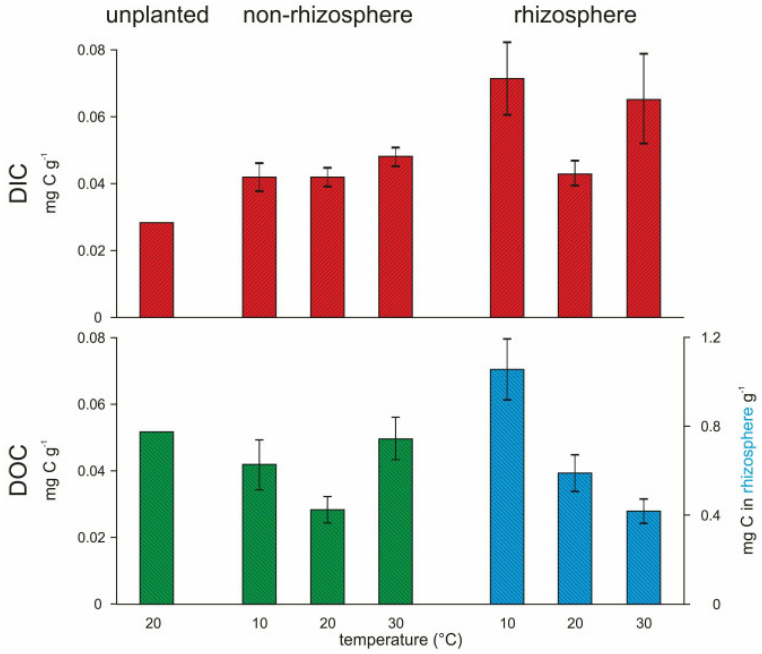

Fig. 4-3: Comparison of DIC and DOC contents in unplanted, non-rhizosphere and rhizosphere loess, normalized to 1 g loess. Only values for unsealed plant pots are shown.

DOC contents did not change significantly with time for each treatment and were not significantly different between temperatures in non-rhizosphere loess (0.01 – 0.08 mg g^{-1} loess), but decreased significantly in rhizosphere loess with increasing temperatures (1.06 ± 0.14 vs. 0.59 ± 0.09 vs. 0.42 ± 0.06 mg g^{-1} loess; Fig. 4-3b, c).

4 Discussion

4.1 Influence of temperature on $CaCO_3$ recrystallization rates

At each of the three temperatures, amounts of recrystallized $CaCO_3$ increased approximately in a linear way from the first to the last sampling. This confirmed the assumption that during the initial stage (weeks – months) of plant growth, constant CO_2 release by roots and rhizomicroorganisms entails constant $CaCO_3$ recrystallization (Kuzyakov et al., 2006; Gocke et al., 2010b). With the graph of the trend curves intersecting the time axis approximately at the date of the first labeling, their slopes corresponded to initial recrystallization rates in loess, which were in the range of 10^{-6} – 10^{-4} day^{-1} and strongly increased with temperature (Fig. 4-1).

Generally, increasing temperature accelerates the adjustment of a reaction equilibrium between reactants (Krauskopf and Bird, 1995), an effect which was not assessed here. However, it was shown that initial $CaCO_3$ recrystallization rates themselves are strongly influenced by temperature: in non-rhizosphere loess, rates were one order of magnitude higher at 30 °C compared with 10 °C (Fig. 4-1). This is about 2.5 times stronger than it could be expected by the common Q_{10} of about 2 (Davidson et al., 2006). This can partly be explained by the character of the $CaCO_3 – CO_2 – HCO_3^-$ system: at higher temperature, low solubility of CO_2 entails decreasing $CaCO_3$ solubility, thus promoting $CaCO_3$ precipitation (Arkley, 1963), if dissolved Ca^{2+} and CO_3^{2-} are present. On the other hand, a considerable amount of $CaCO_3$ has to be dissolved before precipitation, which is promoted by increasing amounts of dissolved CO_2. Decreasing solubility of CO_2 and to lesser extent of $CaCO_3$ with increasing temperature (Krauskopf and Bird, 1995) predicts lower recrystallization rates at high temperatures and *vice versa*, which disagrees with our findings.

Quite the contrary, CO_2 concentrations in the planted microcosms were higher at 20 and 30 °C compared to 10 °C (Fig. 4-2). Our results suggest considerable influence of biological activity on recrystallization rates, as plant growth, root respiration and exudation and microbial respiration are promoted by increasing temperature (Orlov et al., 1997).

It was shown before that recrystallization rates strongly depend on CO_2 concentrations, with higher values at high CO_2 concentration and *vice versa*. The recrystallization rates in loess without plants increased strongly in the range between atmospheric CO_2 concentration (0.04 v-%) and ~ 2 v-%, whereas saturation was reached in the range between 2 and 3 v-% (Gocke et al., 2010a). In the present study, however, CO_2 concentration in each treatment was within or above this range (Fig. 4-2), suggesting that it cannot be the sole reason for the exponential increase of $CaCO_3$ recrystallization rates with temperature. DIC contents did not show contrasting values for different temperatures. This indicates that the maximal possible amount of CO_3^{2-} was dissolved in pore space solution. At the same time amounts of root exudates – as far as reflected by DOC – which could promote $CaCO_3$ dissolution by pH alteration, were not different between the temperatures (Fig. 4-3b).

The influence of a further factor on $CaCO_3$ precipitation was discussed in Gocke et al. (2010b): water uptake by roots, leading to an increase of Ca^{2+} and HCO_3^- concentrations in soil solution to oversaturation close to the root surface, which finally causes precipitation of secondary $CaCO_3$, in special form of pedogenic carbonates – rhizoliths (calcified roots). Comparing two times higher $CaCO_3$ recrystallization rates in rhizosphere compared to non-rhizosphere loess, it was concluded that this difference is partly caused by the roots' transpirational pull. In the present study, daily watering of plants to keep equal moisture in all treatments caused different variations of water content, depending on water consumptions and thus on temperature. Lowest variation occurred at 10 °C (65 – 70% WHC) and highest at 30 °C (25 – 70% WHC; Fig. 4-4). The reason for this is that transpiration of plants strongly increases by rising temperature, partly because of the increase of water pressure deficit. Figure 4-4 shows the variations in water content caused by the plants' water uptake at three temperatures. In contrast to CO_2 concentrations which increased only from 10 °C to 20 °C,

but not from 20 °C to 30 °C, the water uptake presumably exerted main influence on $CaCO_3$ recrystallization rates, because strongly alternating wet and dry conditions promote dissolution and precipitation of $CaCO_3$ and consequently formation of secondary carbonates (Becze-Deák et al., 1997; Borchardt and Lienkaemper, 1999).

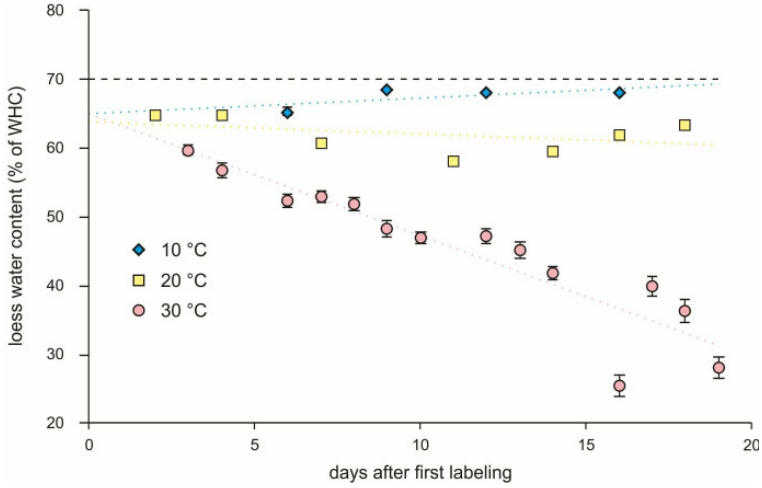

Fig. 4-4: Minimum loess moisture levels, resulting from different plant transpiration rates at 10, 20 and 30 °C. Difference between 70% of WHC (dashed line) and dotted lines reflects daily variation of water content before gravimetric adjustment to 70% of WHC.

Generally, higher temperature leads to enhanced evapotranspiration (Lal and Kimble, 2000). However, the influence of vegetation on secondary $CaCO_3$ precipitation in general, as well as on formation of calcretes (indurated accumulation horizons of pedogenic carbonate) was often underestimated until the late 1970s (Goudie, 1996). Since then, dissolution of soil $CaCO_3$ was attributed to biogenic CO_2, but reprecipitation was still thought to be controlled by abiotic processes like pCO_2 decrease or evaporation of the soil solution (Salomons and Mook, 1976). In subsequent studies, for calcrete formation the biotic process of plant transpiration was considered to have stronger effects on suction pressure in soil compared to abiotic evaporation (Klappa, 1983). Schlesinger et al. (1987) showed for desert soils of New Mexico that roots can be responsible for a removal of up to 72% of incident precipitation by plant transpiration and concluded a significant influence of vegetation on $CaCO_3$ accumulation in soil. Similarly, Jones and Ng (1988) clearly showed the considerable influence of plants on secondary $CaCO_3$ precipitation in the vadose zone: While roots control the rhizosphere moisture level by evapotranspiration, associated microorganisms can enforce cementation, both processes leading to formation of rhizoliths. Following studies recognized the importance of mass flow to the roots, induced by transpiration, for pedogenic carbonate precipitation in general (e.g. Cramer and Hawkins, 2009).

In the present study, the influence of the water effect on $CaCO_3$ recrystallization rates was obvious not only present in the rhizosphere, where direct influence of living roots was expected to create temperature-dependent results, but even in loess distant to roots. There, the difference between different temperatures had an even stronger effect (factor ~ 11 between rates at 10 and 30 °C) compared to rhizosphere loess (factor ~ 4).

Comparison of recrystallization rates in rhizosphere and non-rhizosphere loess revealed ratios in the range between 20 and 100, with highest values at 10 °C (72), lower values at 20 °C (49) and lowest ratio at 30 °C (24; Fig. 4-1). We suggest that increased CO_2 concentration, higher gas viscosity and thus stronger CO_2 diffusion at higher temperature (White and Oostrom, 1996) led to enlarged volume of influence of rhizosphere respiration. This might have somewhat diminished the contrast between non-rhizosphere and rhizosphere loess with increasing temperature.

In contrast to the present findings, Gocke et al. (2010b) reported for with wheat and ryegrass that $CaCO_3$ recrystallization rates were merely twice as high in rhizosphere loess when compared to non-rhizosphere loess. One possible cause for stronger differences between rhizosphere and non-rhizosphere recrystallization rates in the present study might be the impact of rhizosphere sampling: In consequence of irregular distinction between rhizosphere and non-rhizosphere material and different moisture levels at the sampling dates, varying portions of non-rhizosphere loess may be included in rhizosphere samples. Smallest absolute amounts of rhizosphere loess (8 ± 1 g) were obtained at 10 °C and highest amounts (23 ± 2 g) at 30 °C, meaning that potentially lowest amounts of non-rhizosphere loess were sampled as rhizosphere material at 10 °C and highest portion at 30 °C. A possible 'contamination' of rhizosphere loess with non-rhizosphere loess during sampling might also explain decreasing DOC contents in rhizosphere loess with increasing temperature (Fig. 4-3c). In spite of these uncertainties, our findings clearly demonstrate the strong influence of living roots on $CaCO_3$ recrystallization rates in soil.

4.2 Effect of temperature on $CaCO_3$ recrystallization periods

Based on $CaCO_3$ recrystallization rates in non-rhizosphere and rhizosphere loess, we calculated the periods necessary for complete (95%) recrystallization of loess $CaCO_3$ by formation of secondary $CaCO_3$. Assuming repeated dissolution and reprecipitation of both primary and secondary $CaCO_3$ prior to formation of concretions, the amount of secondary $CaCO_3$ is expected to increase in an exponential way (Kuzyakov et al., 2006; Gocke et al., 2010a, b).

Using a typical growing season of 4 months for maize, the plant used in the experiment, recrystallization periods of 8610 years, 4330 years and 800 years were calculated in non-rhizosphere loess for treatments with 10, 20 and 30 °C, respectively. Thus, the strong influence of increasing temperature on $CaCO_3$ recrystallization was reflected not only by increasing rates but also by corresponding one order of magnitude decreasing length of recrystallization periods. However, at equal amounts of precipitation, increasing temperature leads to decreasing length of the growing season, as less water is available to the roots. Recalculation of recrystallization periods with growing seasons of 6 months at 10 °C, 4 months at 20 °C and 3 months at 30 °C showed that 5740 years, 4330 years and 1060 years would be necessary for 95% recrystallization of loess $CaCO_3$ (Fig. 4-5a). In rhizosphere loess, significantly higher $CaCO_3$ recrystallization rates yielded considerably shorter time periods of 80, 90 or 45 years, if temperature-dependent growing seasons are assumed as above. These

periods are even shorter if secondary $CaCO_3$ concretions form around the roots (rhizoliths; Klappa, 1980; Pustovoytov and Terhorst, 2004; Gocke et al., 2010b), preventing further dissolution and reprecipitation of $CaCO_3$. In this case, linearly increasing amounts of recrystallized $CaCO_3$ would lead to complete recrystallization within 25, 30 or 15 years (Fig. 4-5b). This agrees with the assumption that rhizolith formation takes place during the life span of shrubs or trees (Gocke et al., submitted). However, the majority of paleoenvironmental studies using stable isotope composition of pedogenic carbonates were performed on those concretion types which formed distant from roots, e.g. coatings (Wang and Anderson, 1998; Pustovoytov et al., 2007a) and nodules (Pendall et al., 1994; Deutz et al., 2001; Dworkin et al., 2005; Quade et al., 2007). The time frame of their formation or recrystallization is best reflected by extrapolation of $CaCO_3$ recrystallization rates from non-rhizosphere loess. This means that i) $CaCO_3$ in grassland soils is completely recrystallized within 10^2 – 10^3 years and that ii) formation and recrystallization of secondary $CaCO_3$ requires shorter periods under high temperatures compared to lower temperature.

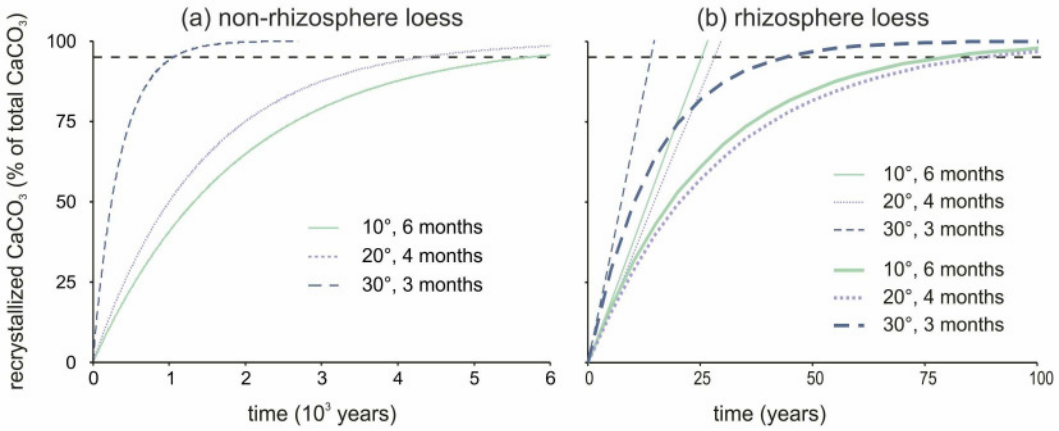

Figure 4-5: Comparison of $CaCO_3$ recrystallization periods extrapolated from recrystallization rates at 10, 20 and 30 °C (note different scale on X axes). (a) Periods necessary for complete recrystallization of total $CaCO_3$ in non-rhizosphere loess, assuming temperature-dependent length of growing season, i.e. as an example 6 months at 10 °C, 4 months at 20 °C and 3 months at 30 °C. (b) Periods necessary for complete recrystallization of total loess $CaCO_3$ in rhizosphere loess, depending on formation of rhizoliths (straight lines) or repeated recrystallization (exponential lines). Black dashed lines represent the level of 95% recrystallization.

The majority of studies on pedogenic carbonates was done in regions of arid and semiarid climatic conditions, e.g. in the deserts of Southwestern USA (e.g. Machette, 1985; Marion, 1989) and Western Asia (e.g. Pustovoytov et al., 2007a). Comparing the soil moisture contents at planted and at harvested plots, Schlesinger et al. (1987) already hypothesized the substantial role that roots might play for rate and depth of carbonate precipitation in arid soils by controlling the amount and movement of soil water and its Ca^{2+} and HCO_3^- concentration. Our findings show the significant indirect effect of ambient temperature on $CaCO_3$ recrystallization periods by controlling plant growth and associated biological processes like respiration and water uptake.

Under natural conditions, mixed effects of temperature combined with rainfall on $CaCO_3$ recrystallization rates and periods occur. As an example, under highly arid conditions, even shorter growing seasons should reinforce the shortening effect of increasing temperature on

recrystallization periods, and therefore further diminishing the contrast between slow $CaCO_3$ recrystallization at low temperature and *vice versa*. Additionally, cold arid climate decreases biological activity in soil (Orlov et al., 1997), and low CO_2 concentration from reduced rhizosphere respiration will entail lower $CaCO_3$ recrystallization rates corresponding to longer recrystallization periods (Gocke et al., 2010a).

The direct or indirect influence of further factors on carbonate recrystallization in soil, e.g. soil moisture or primary $CaCO_3$ content, should be investigated. Together with the data presented, these results might be useful to develop a model for prediction of time spans of pedogenic carbonate formation and recrystallization under various climatic conditions.

4.3 Consequences for paleoenvironmental studies

Since recovery of the C isotopic relation between pedogenic carbonates and soil CO_2 (Cerling, 1984), carbon isotope composition of pedogenic carbonates has been used in numerous studies to assess paleoenvironmental conditions and age of pedogenesis. This requires long-term preservation of the isotopic signal, i.e. the soil has to act as a closed system, which is not true for all soils. Recrystallization and isotopic re-equilibration of existing pedogenic $CaCO_3$ with younger soil CO_2 may entail overprinting of the original isotope composition (Pendall et al., 1994; Amundson et al., 1994). For soils that lack postsegregational contamination of pedogenic carbonates, methodological resolution of paleoenvironmental and chronological studies is nevertheless restricted by the time frame of pedogenic carbonate formation (Royer et al., 2001). This time frame – 10^3 to 10^4 years according to Cerling (1984, 1999) – exceeds instrumental precision (IRMS for $\delta^{13}C$, AMS for radiocarbon dating). The exact time scale of pedogenic carbonate formation could not be assessed under field conditions so far (see chapter 1).

In the present study, we extrapolated $CaCO_3$ recrystallization rates obtained from ^{14}C labeling of plants under controlled conditions to estimate the periods necessary for complete (95%) $CaCO_3$ recrystallization by formation of secondary $CaCO_3$. Certain factors influencing the time scale of pedogenic $CaCO_3$ formation and recrystallization in soil could not be considered: Using plant pots that hamper CO_2 exchange with atmospheric air (also in unsealed pots), initial recrystallization rates were slightly raised by high CO_2 concentration in the loess-root compartment as well as rather humid conditions. Consequently, ^{14}C labeling of plants under controlled conditions overestimated $CaCO_3$ recrystallization rates and underestimated recrystallization periods. Under natural conditions, somewhat lower soil CO_2 concentration should tend to slow down the process of secondary carbonate formation and recrystallization. Moreover, in arid regions where pedogenic carbonates typically form, reduced rooting density and rooting depth have to be considered, potentially leading to lower $CaCO_3$ recrystallization rates. Further, our results are valid only for soils developed on calcareous sedimentary parent material. In soils developed on more compact primary $CaCO_3$ or non-calcareous parent material, recrystallization rates are limited by lower $CaCO_3$ dissolution rates, rates of Ca^{2+} influx from external sources or weathering rates of Ca-bearing minerals (Birkeland, 1999).

Despite these restrictions, the estimated periods indicate the temporal order of magnitude of pedogenic carbonate recrystallization: Using plant pots of 7 cm height, we simulated the conditions in the uppermost 7 cm of a soil profile. In natural soil profiles, radiocarbon ages of pedogenic carbonate in this depth represent the time of recrystallization of secondary $CaCO_3$ rather than the time of its formation (Kuzyakov et al., 2006). Our findings are comparable to recrystallization periods calculated by Kuzyakov et al. (2006) on the basis of radiocarbon data from several soil profiles (Becker-Heidmann et al., 1996). These periods, ranging between some decades and thousands of years, were shorter at high temperatures (26 °C: 130 years) compared to lower temperatures (15 °C: 2500 years). As such detailed radiocarbon data are scarce for upper soil horizons, it was not possible to compare our estimated recrystallization periods to field data obtained from chronosequence studies with different temperature but constant rainfall. However, studies on pedogenic carbonate coatings grown on the lower side of clasts point at a similar direction: Pustovoytov (2003) compared $CaCO_3$ coating growth rates from soil profiles at Middle and Southeastern Europe, Western Asia, Russia and North America. These growth rates seemed to be slightly promoted by increasing temperature. Carbonate coatings form in gravelly parent material, while results from the present study were obtained from secondary carbonates formed in loess. Despite unequal formation and accumulation mechanisms in both types of parent material (Gile et al., 1966), this finding from Pustovoytov (2003) agrees with our results from controlled conditions.

Simple mass balance calculation (Kuzyakov et al., 2006) demonstrated that contamination of secondary $CaCO_3$ of Holocene age even with a small portion of primary $CaCO_3$ can entail strong overestimation of the true radiocarbon age. Therefore, the time necessary for recrystallization represents the upper limit of the resolution of paleoenvironmental studies based on isotope composition of pedogenic carbonates. For pedogenic carbonates which were not formed by encrustation of roots (i.e. rhizoliths), the precision of such paleoenvironmental reconstructions cannot be better than thousands of years, depending on length of the growing season, which in turn is affected by temperature.

5 Conclusions

Based on isotopic exchange of primary loess $CaCO_3$ with $^{14}CO_2$ released by root and rhizomicrobial respiration of ^{14}C labeled plants, initial $CaCO_3$ recrystallization rates in the range of 10^{-4} day^{-1} – 10^{-6} day^{-1} were estimated. Plants grown at 10 °C showed lowest recrystallization rates, while higher rates were obtained from plants grown at 20 °C. Fastest recrystallization occurred under plants grown at 30 °C, with recrystallization rates one order of magnitude higher compared to those at 10 °C. This strong increase was attributed to the boosting effect of temperature on shoot and root growth and associated rhizosphere processes especially root and rhizomicrobial respiration. Additionally, enhanced moisture contrast resulting from the plants transpirational pull and, to a smaller extent, increased root and rhizomicrobial respiration under high temperature led to reinforced dissolution and reprecipitation of $CaCO_3$, as a result of enhanced moisture contrast and increased CO_2 concentrations. This showed the enormous significance of vegetation for pedogenic carbonate

formation and recrystallization and demonstrated that the temperature as a climatic factor controls the recrystallization indirectly – by biotic processes.

For extrapolation of these initial $CaCO_3$ recrystallization rates the effect of temperature on length of the growing season has to be considered. This diminishes to some extent the contrast between low and high temperatures when regarding the periods necessary for complete recrystallization. In loess distant to living roots, the time frame of recrystallization was 5740 years at 10 °C, 4330 years at 20 °C and 1060 years at 30 °C if assuming growing seasons of 6, 4 and 3 months, respectively. These estimated periods reflect the minimum times as the experimental layout leads to overestimated recrystallization rates. These findings indicate that in grassland soils at least 10^3 years are necessary for complete (95%) recrystallization of loess $CaCO_3$ and formation of secondary $CaCO_3$. This time frame represents the upper limit of the temporal resolution of paleoenvironmental studies based on isotope composition of pedogenic carbonates.

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Study 5: Pedogenic carbonate formation: recrystallization vs. migration – process rates and periods assessed by ^{14}C labeling

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Abstract

Under arid to semihumid climatic conditions, dissolution of primary carbonate and recrystallization with carbon (C) from soil CO_2 leads to accumulation of significant amounts of pedogenic (secondary) carbonate. Most soils of arid and semiarid regions contain a carbonate accumulation horizon, the depth of which is related to climatic conditions and properties of parent material. It remains unclear, whether this carbonate is migrated from the upper horizons before or after recrystallization with soil CO_2. The aims of this study were to determine recrystallization rates during initial pedogenesis and to localize the accumulation depth of secondary carbonate based on C isotopic exchange during secondary carbonate formation in an experiment with alternating moisture conditions. Maize grown on 1 m high loess-filled columns was pulse labeled in $^{14}CO_2$ atmosphere every 3 weeks. After six months, amounts of recrystallized $CaCO_3$ were determined in 5 cm segments, based on ^{14}C respired in the rhizosphere and subsequently incorporated into newly formed secondary carbonate. More than 80% of recrystallized carbonate ($Ca^{14}CO_3$) were leached from the uppermost 15 cm of the loess column, and more than 70% of total secondary carbonate were accumulated in a depth between 15 and 50 cm. Based on the recrystallization rate calculated for the uppermost 15 cm of the loess column ($1.77 \pm 0.26 \cdot 10^{-5}$ day^{-1}), between 300 and more than 1,700 years are necessary for complete decalcification of the upper 15 cm. Our modeled data fit with time intervals for decalcification and formation of calcic horizons under more humid conditions.

Keywords: soil inorganic carbon, loess, carbonate recrystallization, carbon isotopic exchange, carbonate accumulation

1 Introduction

Pedogenic (secondary) carbonates are a characteristic feature of soils of arid to semihumid climatic conditions [*Jenny*, 1994; *Birkeland*, 1999; *Eswaran et al.*, 2000], and contribute as soil inorganic carbon (SIC) 30–40% to the global soil carbon stock [*Batjes*, 1996; *Eswaran et al.*, 2000]. Under certain conditions (shifting of humid climatic zones, soil acidification), the SIC pool can be leached [*Lapenis et al.*, 2008] and incorporated in the global C cycle, thus contributing to the greenhouse effect [*Lal and Kimble*, 2000].

In soils and paleosols not affected by groundwater, secondary carbonates provide several proxies for reconstruction of paleoenvironmental conditions: stable carbon and oxygen isotopic composition ($\delta^{13}C$, $\delta^{18}O$), morphology, depth of carbonate accumulation, which can be used to assess paleovegetation, -temperatures, -precipitation, former atmospheric pCO_2, and soil age (by $\Delta^{14}C$).

As pedogenic carbonates are precipitated in isotopic equilibrium with soil CO_2 [*Cerling*, 1984; *Nordt et al.*, 1996], released mainly by root and rhizomicrobial respiration [*Amundson et al.*, 1998], their carbon stable isotopic composition ($\delta^{13}C_{carb}$) reflects the photosynthetic pathway of the vegetation present during their formation [*Cerling et al.*, 1989; *Amundson et al.*, 1989; *Cerling and Quade*, 1993]. Therefore, pedogenic carbonates have served as a tool for reconstructing paleovegetation in a number of studies [*Wang et al.*, 1996; *Pustovoytov et*

al., 2007; *Monger et al.*, 2009]. In contrast, the oxygen stable isotopic composition of secondary carbonates ($\delta^{18}O_{carb}$) is related to that of meteoric water [*Cerling*, 1984] and of soil moisture [*Quade et al.*, 1989; *Cerling and Quade*, 1993]. Through their linkage to meteoric water, $\delta^{18}O$ values of pedogenic carbonates can provide a record of paleotemperatures [*Quade and Cerling*, 1993; *Dworkin et al.*, 2005], precipitation amount [*Wang et al.*, 1996], paleopatterns of atmospheric circulation [*Amundson et al.*, 1996] or a combination of these parameters [*Monger et al.*, 1998].

As shown by several studies, regional environmental conditions prevailing during formation of pedogenic carbonates can be reflected in their morphological occurrence [*Gile et al.*, 1966; *Courty et al.*, 1994; *Khokhlova et al.*, 2001; *Pustovoytov*, 2002; *Khokhlova and Kouznetsova*, 2004], as well as in growth rates of carbonate coatings [*Pustovoytov*, 2003].

Additionally, pedogenic carbonate concretions yield potential for determination of soil age and age of carbonate accumulation by radiocarbon dating [*Amundson et al.*, 1994; *Pustovoytov*, 2003; *Amoroso*, 2006], U/Th dating [*Sharp et al.*, 2003] and thickness of carbonate coatings [*Pustovoytov*, 2003; *Amoroso*, 2006].

In moderate to mature soil profiles, pedogenic carbonates typically occur within one soil horizon [*Gile et al.*, 1966; *Birkeland*, 1999], which is called carbonate accumulation horizon or calcic horizon, abbreviation Bkc, if it meets the criteria of WRB [*FAO/ISRIC/ISSS*, 2006]: $\geq$ 15 cm thickness, $\geq$ 15% $CaCO_3$, $\geq$ 5% more $CaCO_3$ than underlying horizon or $\geq$ 5% secondary carbonate. The depth of the top of the calcic horizon can show a wide range between 0 cm (in some Calcisols) and more than 2 m below the surface (in some modern or relict steppe soils such as Phaeozems), descending deeper when transferring to more humid geographical zones where it is usually located beyond soil profiles. For soil studies, usually Bkc horizons with upper boundary between 0.2 and 1 m are relevant [*Arkley*, 1963; *McFadden and Tinsley*, 1985; *Birkeland*, 1999]. This depth is also important for the degree of isotopic reequilibration of carbonate with soil CO_2 [*Pendall et al.*, 1994], the prerequisite for the suitability of pedogenic carbonates as a tool for paleoenvironmental reconstructions based on stable isotopic composition as described above.

Prerequisites for accumulation of soil carbonates are a carbonate-rich soil solution, downward transport of Ca^{2+} and HCO_3^- by this solution, and subsequent precipitation of $CaCO_3$ [*Salomons et al.*, 1978]. In general, depth [*Jenny*, 1994; *Birkeland*, 1999] and rate [*Marion*, 1989] of carbonate accumulation increase with increasing amounts of rainfall. Besides precipitation, the depth of carbonate leaching and of secondary $CaCO_3$ accumulation within the soil profile depends on further climatic conditions like temperature and evapotranspiration [*Arkley*, 1963; *Lal and Kimble*, 2000; *Retallack*, 2005; *Rubio and Escudero*, 2005]. Furthermore, vegetation, relief, surficial run-off, amount of available Ca^{2+} and HCO_3^- ions (either inherited or incorporated from dust or rainfall), depth of water penetration and properties of the parent material influence depth and thickness of carbonate accumulation in soils [*Arkley*, 1963; *Klappa*, 1983; *McFadden and Tinsley*, 1985; *Jenny*, 1994; *Birkeland*, 1999; *Nordt et al.*, 2006]. Moreover, strongly alternating moisture conditions enhance carbonate accumulation by dissolution and downward transport during

wet phases and $CaCO_3$ precipitation in lower parts of the soil profile during dry phases because of increasing saturation of the soil solution with respect to Ca^{2+} and CO_3^{2-}. While contrasting dry and wet seasons likely promote calcification of roots [*Becze-Deák et al.*, 1997], the effect of seasonal distribution of rainfall on leaching and accumulation depth, however, seems to differ [*Yaalon*, 1997; *Rubio and Escudero*, 2005].

In paleosols, depth of carbonate accumulation and morphology stages, describing the increasing level of carbonate impregnation (as established by *Gile et al.* [1966] and expanded by *Machette* [1985]; stages I–VI), have been used for reconstruction of paleoprecipitation (e.g. *Retallack* [2005]; see review by *Sheldon and Tabor* [2009]) and determination of soil age [*Gile*, 1993]. Rates of carbonate accumulation are of great importance for chronosequence studies of soils and paleosols and for estimation of ages of Quaternary deposits [*Birkeland*, 1999]. According to *Lal and Kimble* [2000], rates of accumulation are higher in gravelly soils when compared to non-gravelly soils, i.e. that stage VI is reached more rapid in soils containing solid rock fragments. In a non-gravelly soil, developed e.g. on sediments, accumulation of carbonate in a certain depth can take hundreds of thousands of years [*Gile and Grossman*, 1979].

Main work concerning carbonate accumulation in non-gravelly soils was performed in New Mexico / Southwestern USA, where soils developed mainly on non-calcareous parent materials and the rate of carbonate accumulation is presumed to depend mainly on rates of $CaCO_3$ influx from dust and / or rainfall [e.g. *Gile et al.*, 1966; *Machette*, 1985; *McFadden and Tinsley*, 1985]. In calcareous parent material like loess, in contrast, the rate of pedogenic carbonate buildup is a function of the rate at which originally present $CaCO_3$ can be dissolved and translocated by leaching waters in a soil profile [*Birkeland*, 1999]. Moreover, previous studies assessed the time frame for carbonate translocation and formation of calcic horizon only indirectly, based on soil age and carbonate content [*McFadden and Tinsley*, 1985] or on theoretical calculations of water movement [*Arkley*, 1963]. Very slow processes of carbonate alteration by pedogenic processes [*Kuzyakov et al.*, 2006; *Gocke et al.*, 2010 a, 2010b] rule out determination of these rates in the field. Another uncertainty is that primary and secondary carbonate can not always be distinguished [*Lal and Kimble*, 2000; *Eswaran*, 2000], in particular in soils developed on calcareous sediments like loess, because secondary carbonates are not necessarily recognizable morphologically (e.g. concretions).

Kuzyakov et al. [2006] and *Gocke et al.* [2010b] showed that it is possible to estimate very small amounts of secondary $CaCO_3$ in loess (< 0.1% of total loess carbonate) even after time intervals as short as a few weeks by ^{14}C labeling and isotopic exchange approach. The rate of loess carbonate recrystallization by formation of secondary carbonate was determined in newly formed $CaCO_3$ by quantifying ^{14}C, assimilated by plants in an artificial $^{14}CO_2$ atmosphere and respired by root and rhizomicrobial respiration. Recrystallization rates in the range of 10^{-5} day^{-1} were obtained, i.e. per day 10^{-3}% of the total loess carbonate were recrystallized, leading to complete recrystallization within few thousands of years. These studies, however, did not take into account carbonate translocation by percolating water.

We hypothesize that this labeling and isotopic exchange approach is suitable

- to show in a time of few months that secondary $CaCO_3$ is transported downwards by percolating water and that the carbonate accumulation horizon contains the highest portion of secondary $CaCO_3$ within the soil profile and
- to reveal even very small amounts of secondary carbonate in loess in the initial stage of pedogenesis, when translocation of carbonate by percolating water is not yet measurable in terms of depth-dependant distribution of total $CaCO_3$.

The main aim of the present study was 1) to show the depth-related distribution of secondary carbonate obtained by alternating drying and wetting, and 2) to visualize this relation in the initial stage of pedogenesis on a calcareous parent material. Further aims were

- quantification of leaching of carbonates from the upper layers and of accumulation in lower layers and
- estimation of the period necessary for complete decalcification of upper layers. As carbonate migration from upper layers is the prerequisite for formation of an accumulation horizon, this time period roughly indicates the maximum age of calcic horizons in loess.

2 Materials and methods

2.1 Experimental setup

To test the effect of carbonate leaching on the depth of secondary $CaCO_3$ accumulation, maize (*Zea mays* [L.], cv. Tassilo) was grown on loess. This plant species was chosen because of its deep-rooting properties. The loess was sampled at the open cast mine of the HeidelbergCement AG, Nussloch (SW Germany, N 49°18'41.1", E 8°43'37.2", 211 m a.s.l.) from a depth of 15 m below present surface. C_{carb} content of the loess was 32.9 mg g^{-1}, corresponding to a $CaCO_3$ content of 274 mg g^{-1}. The loess-paleosol sequence of Nussloch was deposited mainly during the last glacial-interglacial cycle [*Antoine et al.*, 2001]. Instead of soil, loess was used as plant growing medium because it is not influenced by modern pedogenic processes like humification, leaching or calcification. This means that first, the loess $CaCO_3$ is primary, i.e. not recrystallized, and shows an even, diffuse distribution as well as small, uniform grain size; second, C_{org} content of the loess was low (0.3 mg g^{-1}; *Wiesenberg et al.* [2010]). Hence, CO_2 concentration and fluxes from root and rhizomicrobial respiration during the experiment were not altered considerably by CO_2 released from microbial decomposition of previously present organic matter, as would be in the case of soil. In summary, the experiment simulated conditions of initial soil formation in sedimentary calcareous parent material.

As plant pots, acryl glass tubes (Plexiglas® XT; Röhm, Germany) with an inner diameter of 46 mm and a height of 1 m were used. Each tube had one opening at the top for plant growth and three sidewise inlets, on at the bottom and two at the top (Figure 5-1). For convenient sampling (see below), the acryl glass tubes were lined with polyethylene terephthalate foil (Nalophan®; Kalle, Germany) with an inner diameter of 43 mm. Each lined

tube was filled with 1,600 g of air-dried and sieved (2 mm) loess. The height of each loess column was 85 cm, resulting in a loess density of 1.3 g cm^{-3}.

Ten maize plants were grown on loess under controlled laboratory conditions, one plant per acryl glass tube. The maize seedlings were first germinated on wet filter paper and transferred to the plant pots after 3 days. For the introduction of microorganisms, 10 ml of soil extract from a Haplic Luvisol (developed from loess) were added to each plant pot. The plants were grown at 14/10 h day/night periods and light intensity of 300 µmol m^{-2} s^{-1}. For nutrient supply, the plants were treated with Hoagland nutrient solution [*Hoagland and Arnon*, 1950] which was adapted to the high amount of $CaCO_3$ in loess (see *Gocke et al.* [2010b]). The applied nutrient solution contained 138, 62 and 469 µg ml^{-1} of N, P and K, respectively.

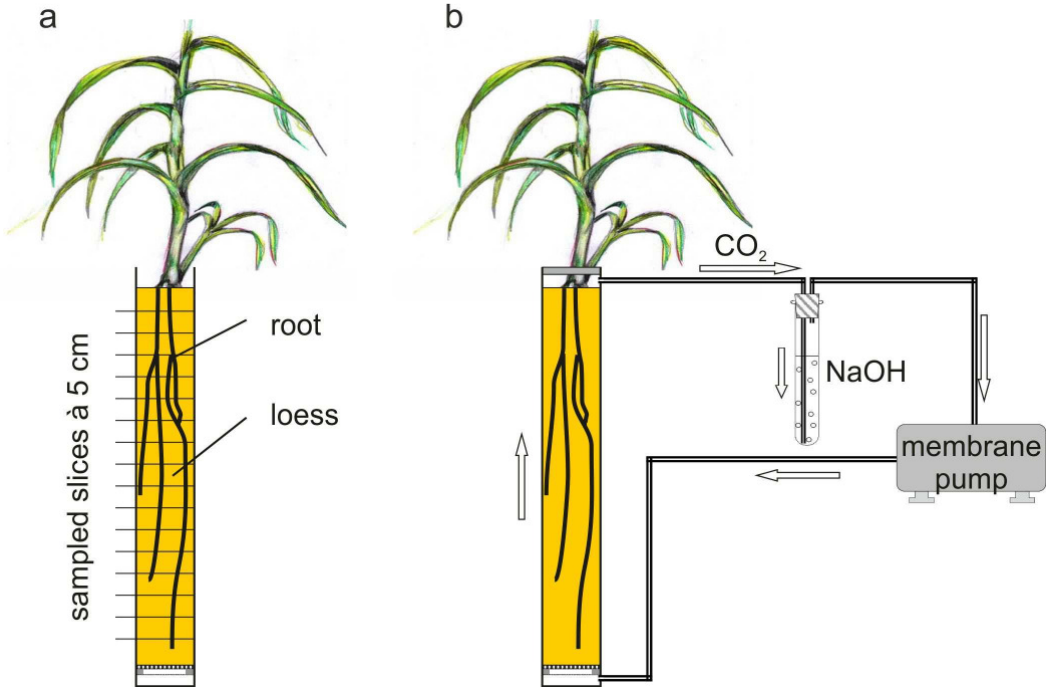

Figure 5-1. a) Experimental layout and b) trapping of respired CO_2, shown for sealed pots.

Twenty-one days after planting, five of the plant pots were sealed with non-toxic two-component silicone rubber (Tacosil 145, Thauer & Co., Germany) between roots and shoots and the seal was tested for air leaks. The purpose of the sealing was to separate the air in the root-loess compartment from the atmospheric air and thus to avoid loss of ^{14}C labeled and total CO_2 released by root and rhizomicrobial respiration. The other five plant pots were not sealed to allow air exchange between atmosphere and the root-loess compartment, thus simulating natural conditions. Directly before applying the first ^{14}C pulse label, every plant pot was flushed with fresh air to remove all previously accumulated CO_2 from root and rhizomicrobial respiration. For this purpose, the bottom inlet of each pot was connected to the output of a membrane pump (Type SMG4, Gardner Denver Thomas GmbH, Germany) for several hours. Afterwards, the inlets of the five sealed plant pots were plugged.

2.2 ^{14}C pulse labeling

Twenty-one days after planting, the 10 maize plants were labeled for the first time in an airtight acryl glass chamber ($0.5 \cdot 0.5 \cdot 1.6$ m^3) equipped with an internal ventilation unit for equal distribution of the ^{14}C labeled CO_2. The labeling procedure was described in detail elsewhere [*Kuzyakov et al.*, 2006; *Gocke et al.*, 2010b; *Fischer et al.*, 2010]. Briefly, the chamber was connected by tubings to a flask containing 10 ml of diluted $Na_2{}^{14}CO_3$ solution (ARC Inc., USA) with 407 kBq per plant pot. $^{14}CO_2$ was released by adding 3 ml of 5 M H_2SO_4 to the label solution and was pumped through the chamber in a closed cycle for 10 min by a membrane pump. The plants were labeled in $^{14}CO_2$ atmosphere during 4 h. The CO_2 compensation point is reached for C_4 plants already after 30 min [*Kuzyakov and Cheng*, 2004], however we chose a longer time period to allow for complete assimilation of the ^{14}C labeled CO_2. After that time, the air of the chamber was pumped through 15 ml of 1 M NaOH to remove the remaining unassimilated CO_2. The chamber was opened and the plants continued growth under normal conditions.

The plants were labeled 3 times in 3 week intervals. One week after the third labeling, plants were harvested and new maize seedlings were planted on the loess. Similar to the first plants, these were grown for 3 weeks, then labeled 3 times in time intervals of 3 weeks. In total, 3 maize plants grew in each acryl glass tube consecutively. The experiment thus consisted of three cycles of plant growth (named A, B and C), with each of them lasting 10 weeks and comprising 3 labelings.

2.3 Moisture conditions

Nutrient solution and water were always added from the top of the acryl glass tubes to simulate natural rainfall. In the following, all indications for the water level in loess-root compartments refer to the uppermost 60 cm of the loess column. The first 2 weeks after planting, loess moisture was held at 70% of water holding capacity (WHC = 28% of loess weight), i.e. 220 ml. All of the 10 plants always received the same volume of nutrient solution at one time. The individual difference to the total water loss was added as de-ionized water.

Seven days before the first labeling, loess moisture was decreased until it reached a minimum level of 30–35% of WHC, corresponding to 94–110 ml. To obtain a maximum effect of carbonate migration on the depth of secondary $CaCO_3$ accumulation, the water level was raised to 70% of WHC again 21–22 hours after the beginning of each labeling. This time period was chosen because release into rhizosphere of CO_2 recently assimilated by maize plants is highest in the first few hours and shows a strong continuous decrease at least within the first 3 days [*Kuzyakov and Cheng*, 2004].

Between the labelings, the water level of the plant pots was adjusted gravimetrically to 70% of WHC only when it reached the minimum level of 30% of WHC, corresponding to a water content of 94 ml. The intervals between two waterings decreased with increasing age and size of the maize plants. The time lags were between 2 and 8 days, depending on the plants' water loss by evapotranspiration. Due to differing plant growth, one cycle of plant growth comprised 12, 9 and 19 wettings for cycle A, B and C, respectively.

In total, moisture conditions corresponded to daily precipitation of 18.5 ± 2.3 mm.

2.4 Sampling and analyses

After the labeling, ^{14}C activities in the residue of the label $Na_2{}^{14}CO_3$ solution and in unassimilated CO_2 trapped in NaOH were measured on 1 ml aliquots mixed with 2 ml of scintillation cocktail (Rotiszint EcoPlus; Carl Roth, Germany). The ^{14}C measurements were done using a liquid scintillation counter (LSC; 1450 LSC & Luminescence Counter (MicroBeta TriLux; Perkin Elmer Inc., USA) standardized by SQP(E) with ^{14}C counting efficiency ≥ 70% and measurement error ≤ 3.5%.

In contrast to previous studies [*Kuzyakov et al.*, 2006; *Gocke et al.*, 2010b], root-respired CO_2 was removed not only directly prior to the subsequent labeling. Instead, CO_2 accumulated in the loess-root compartment was pumped out in one week intervals and analyzed for ^{14}C and total C (C_t) analyses. This time frame was chosen because the time intervals between the individual ^{14}C pulses of 3 weeks were too long for roots in sealed plant pots to grow without fresh air supply. Most of the recently (thus ^{14}C labeled) assimilated CO_2 is respired into rhizosphere within the first 3 days [*Kuzyakov and Cheng*, 2004], and the C isotopic exchange between available CO_2 and $CaCO_3$ is completed within less than 4 days [*Gocke et al.*, 2010a]. Hence, 7 days after the labeling, most of the ^{14}C tracer available in loess air CO_2 was already incorporated into loess carbonate by recrystallization.

One, two and three weeks after each labeling, CO_2 from root and rhizomicrobial respiration of every plant pot was pumped through 15 ml of 1 M NaOH for 90 min by a membrane pump. For the sealed plant pots, CO_2 trapping was done in a closed system (Figure 5-1b). ^{14}C activity of respired CO_2 was measured on 1 ml aliquots of NaOH as described above. Total carbon content of respired CO_2 was determined by precipitating trapped CO_2 with 1 M $BaCl_2$ solution. NaOH was then titrated with 0.01 M HCl against phenolphthalein [*Zibilske*, 1994]. CO_2 concentration in the plant pots was calculated from the amount of CO_2-C trapped in NaOH. After removing the rhizosphere CO_2, each plant pot was flushed with atmospheric air for 20 min. Afterwards, inlets of the sealed pots were plugged again, and the plants either continued growth under normal conditions or were labeled.

At the end of the experiment, the lined acryl glass tubes containing loess and roots were frozen. Afterwards, foil liners with loess and roots were pulled out and cut into 5 cm slices from the top to the bottom (17 slices per plant pot; Figure 5-1a). After thawing of the samples, roots were removed from loess in each slice by tweezers and washed with 50 ml of de-ionized water. Immediately afterwards, loess from each slice was carefully mixed and water content was determined gravimetrically: The wet samples were weighed, dried until weight constancy, and weighed again. Loess moisture is expressed as mass-%. Three grams of loess were washed with 10 ml of de-ionized water to remove dissolved inorganic and organic carbon (DIC and DOC). Loess and root samples (each of them 85 in total) as well as shoot biomass from the harvested plants (30 in total) were dried at 60 °C and grinded in a ball mill (MM200, Retsch, Germany).

To analyze ^{14}C incorporated into plant biomass, total carbon of the plant material was converted into CO_2 by combustion at 800 °C ('Feststoffmodul 1300', AnalytikJena, Germany). The evolved CO_2 was trapped in 8 ml of 1 M NaOH and subsequently analyzed for ^{14}C activity by LSC (see above). The amount of ^{14}C incorporated into solid organic substances (loess C_{org}) was determined in the same way at 550 °C and measured in 5 ml aliquots.

The solution from loess washing, containing dissolved inorganic and organic carbon (DIC, DOC), was measured on 4 ml aliquots for ^{14}C activity in total dissolved carbon and, after release of CO_2 from DIC by adding 0.2 ml of 1 M HCl, in DOC. ^{14}C activity of DIC was calculated by subtracting the ^{14}C in washing water before and after DIC release.

To measure ^{14}C incorporated into loess carbonate, two grams of every dried loess sample were treated with 15 ml of 3 M H_3PO_4. CO_2 evolving from $CaCO_3$ was trapped in 12 ml of 2 M NaOH and analyzed for ^{14}C activity on 6 ml aliquots of NaOH added to 12 ml of scintillation cocktail. This method, tested before with a ^{14}C enriched plant sample, was used instead of combustion (the usual method for soil samples), because combustion would lead to CO_2 release not only from carbonate but also from organic matter accumulated in loess during plant growth. An aliquot of the NaOH containing CO_2 evolved from $CaCO_3$ was titrated (see above) to test whether total $CaCO_3$ content of loess had changed in the individual slices after plant growth as a result of carbonate redistribution.

^{14}C measurements of larger aliquots for loess $CaCO_3$, loess C_{org}, DIC and DOC were performed on a LS 6500 Multi-Purpose Scintillation Counter (Beckman, USA) with ^{14}C counting efficiency $\geq$ 90% and measurement error $\leq$ 4%. The absolute ^{14}C activity was standardized by the H number method, using a ^{137}Cs external standard.

2.5 Calculations and statistical analyses

After each labeling, the portion of ^{14}C assimilated by maize plants ($^{14}C_{ass}$) was calculated as follows:

$$^{14}C_{ass} = {}^{14}C_{input} - {}^{14}C_{res} - {}^{14}C_{NaOH} \quad (1)$$

with $^{14}C_{input}$ as input ^{14}C activity, $^{14}C_{res}$ as ^{14}C activity of the label residue and $^{14}C_{NaOH}$ as ^{14}C activity of unassimilated CO_2 trapped in NaOH.

The ^{14}C specific activity of CO_2 respired by roots and rhizosphere microorganisms was calculated as the ratio of ^{14}C activity ($^{14}C_{CO_2}$) and total C content in respired CO_2 (C_{CO_2}):

$$^{14}C^{sp}_{CO_2} = \frac{^{14}C_{CO_2}}{C_{CO_2}} \quad (2)$$

The main prerequisite of our study was that the ^{14}C specific activities of respired CO_2 ($^{14}C^{sp}_{CO_2}$) and of C incorporated into the loess carbonate by recrystallization are equal. This can be assumed because the study was done with strongly enriched ^{14}C some orders of magnitude higher than natural ^{14}C abundance, making isotopic fractionation negligibly low.

Amounts of recrystallized $CaCO_3$ ($CaCO_{3_{recryst}}$) were calculated according to equation 3, based on ^{14}C activity of loess $CaCO_3$ ($^{14}C_{CaCO_3}$) and ^{14}C specific activity of respired CO_2.

$$CaCO_{3_{recryst}} = \frac{^{14}C_{CaCO_3}}{^{14}C^{sp}_{CO_2}} \quad (3)$$

We used $^{14}C^{sp}_{CO_2}$ from sealed pots, where loss of total and ^{14}C labeled CO_2 was prevented, and $^{14}C_{CaCO_3}$ from open pots, where undisturbed exchange of air inside and outside the pots reflected more likely natural conditions in terms of CO_2 concentration, when compared to sealed pots.

The initial $CaCO_3$ recrystallization rate was calculated as the amount of recrystallized $CaCO_3$ divided by the total amount of carbonate in loess ($CaCO_{3_t}$) and by the time (t) between the first labeling and the final harvesting (182 days):

$$CaCO_3 \text{ recrystallization rate} = \frac{CaCO_{3_{recryst}}}{CaCO_{3_t} \bullet t} \quad (4)$$

Based on the initial recrystallization rate, the time period necessary for complete (95%) recrystallization of the loess carbonate was calculated. Most likely, an exponential decrease of the remaining primary $CaCO_3$ can be assumed [*Kuzyakov et al.*, 2006; *Gocke et al.*, 2010b] because of repeated recrystallization of both primary and secondary carbonate. As high CO_2 concentration in soil, an important factor for carbonate recrystallization [*Gocke et al.*, 2010a], occurs mainly during the growing season, the length of this period (GS; in days $year^{-1}$) was included in Equation 5 to estimate the amount of $CaCO_3$ recrystallized under field conditions ($CaCO_{3_{recryst}}(t)$):

$$CaCO_{3_{recryst}}(t) = 100 \bullet (1 - e^{-t \bullet rate \bullet \frac{GS}{365}}) \quad (5)$$

Mean values and standard errors of the mean are presented in figures. The sample set was tested for significance of differences using one-way ANOVA with a significance level of $\alpha = 5\%$, followed by post hoc LSD test. Statistical analysis was carried out using STATISTICA for Windows (version 7.0, StatSoft Inc., Tulsa, USA).

3 Results

3.1 Plant biomass and loess moisture

Total amounts of dry shoot biomass were significantly different between the three cycles of plant growth, with smallest amounts in the second and largest amounts in the last cycle (Table 5-1), but did not differ significantly between open and sealed plant pots, except for the last sampling.

Table 5-1
Dry biomass of plants (g). Mean values ± SEM, n = 5.

	shoots 1. sampling	shoots 2. sampling	shoots 3. sampling	roots (total) 3. sampling
open	6.9 ± 0.2	4.7 ± 0.2	9.8 ± 0.5	11.6 ± 0.7
sealed	7.0 ± 0.4	5.0 ± 0.5	11.7 ± 0.7	n.d.

At the end of plant growth (30 weeks), the loess was completely rooted from top to bottom, however with largest amounts of dry root biomass (2.7 ± 0.4 g) in the uppermost 5 cm of the loess column, immediate decrease to values < 1 g below, and smallest amounts (< 0.4 g) in a depth of 50–85 cm (Figure 5-2a).

Loess moisture, measured after the final harvesting of plants, increased from the top of the loess-root compartment (3.6 ± 0.3%) towards highest values in 40–45 cm (6.6 ± 0.9%), thereunder showed a steep decrease and reached lowest values (mostly < 3%) below 55 cm depth (Figure 5-2a). Although these values of the final water contents are not representative for the whole time of plant growth, the depth-related pattern was in agreement with the calculated depth (60 cm) of maximal moisture penetration at water level of 70% of WHC.

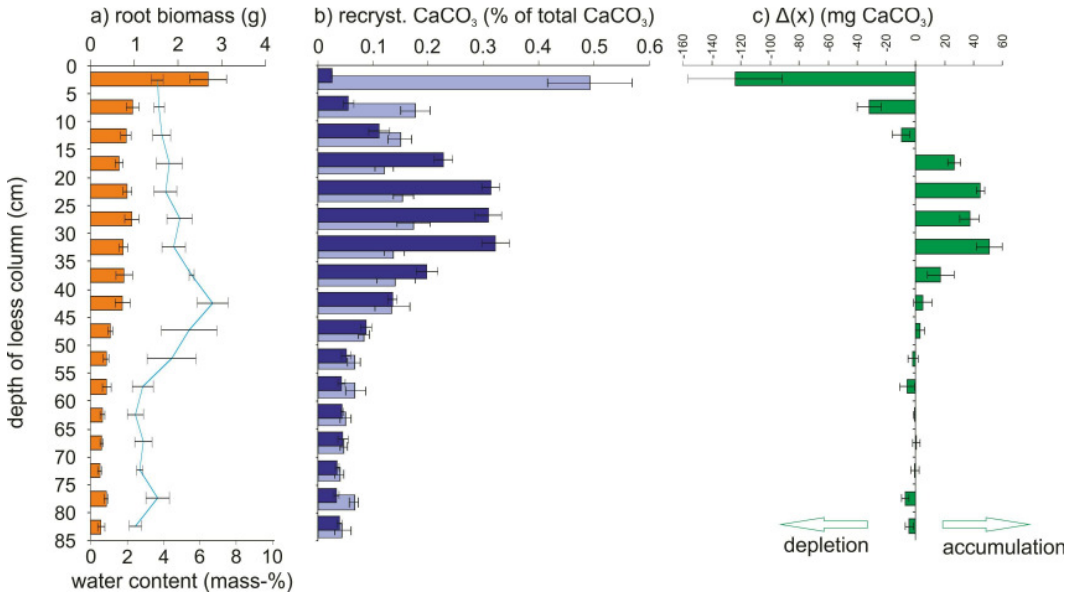

Figure 5-2. Depth-dependant distribution of a) amounts of root biomass and loess moisture measured at the end of the experiment, b) amounts of measured secondary $CaCO_3$, c) theoretically recrystallized amounts of $CaCO_3$, assuming no downward translocation (see Equation 8). Mean values ± SEM, n = 5.

3.2 ^{14}C budget

Averaged for all 9 labelings, 99.6 ± 0.3% of the applied ^{14}C activity (36.63 MBq) were assimilated by the maize plants. The remaining 0.4% (0.15 MBq) were found after labeling in the label residue and in unassimilated CO_2 from the labeling chamber trapped in NaOH.

In open plant pots, recovery of ^{14}C activity in loess, DIC and DOC, plant biomass and CO_2 from root and rhizomicrobial respiration in total was 32–43% of the added ^{14}C label (Table 5-2).

From recovered ^{14}C label, largest portions were found in maize biomass with 74.4 ± 1.0% in aboveground biomass and 18.2 ± 0.8% in roots. In general, only small parts of the ^{14}C label were incorporated into below ground C pools by exudation and respiration of roots: 1.0 ± 0.1% in loess $CaCO_3$, 1.1 ± 0.1% in CO_2 respired by roots and associated microorganisms, 4.0 ± 0.2 in organic matter (loess C_{org}) mainly representing not extractable root debris, and 1.3 ± 0.1% in DIC and DOC (Table 5-2).

Table 5-2
Total ^{14}C recovery after subsequent growth of 3 maize plants, each of them for 10 weeks, in different below- and aboveground pools as percentage of recovered ^{14}C and percentage of input ^{14}C.

	open plant pots
^{14}C [% of ^{14}C recovery]	
Shoots	74.4 ± 1.0
Roots	18.2 ± 0.8
DIC+DOC	1.3 ± 0.1
respired CO_2	1.1 ± 0.1
loess C_{org}	4.0 ± 0.2
loess $CaCO_3$	1.0 ± 0.1
^{14}C [% of ^{14}C input]	
sum of all compartments	37.8 ± 2.0

3.3 Rhizosphere CO_2

From the open plant pots, all above- and belowground C pools (see Table 5-2) were analyzed for ^{14}C, whereas sealed pots were applied only to obtain ^{14}C specific activities of respired CO_2, which are crucial for calculation of amounts of recrystallized $CaCO_3$. At most of the samplings, ^{14}C specific activities were higher in the sealed pots when compared to the open pots. In sealed pots, ^{14}C specific activities were highest at the first CO_2 trapping, i.e. one week after each labeling, and decreased considerably towards the two following CO_2 trappings. This trend can be explained by constant CO_2 release from root and rhizomicrobial respiration on the one hand, and decreasing portions of mobile ^{14}C within the plant (i.e. not incorporated in plant tissue) on the other hand. Moreover, ^{14}C specific activities measured one week after each labeling were highest after the first and lowest after the third labeling within the life span of each plant (Figure 5-3a), as plant growth led to increased storage of assimilated C and thus of ^{14}C in the plant. In open pots, temporal variations of ^{14}C specific activities showed a similar pattern, however with a lower range between lowest and highest values when compared to sealed pots.

CO_2 concentrations in open plant pots, calculated from amounts of CO_2-C trapped in NaOH, were 1.7 ± 0.1% (for water content 70% of WHC: 1.9 ± 0.1%; 30% of WHC:

1.5 ± 0.1%; absolute range 0.6–4.0%; Figure 5-3b). Variations were low when compared to CO_2 concentrations in sealed plant pots (for water content 70% of WHC: 24.0 ± 2.2%; 30% of WHC: 18.5 ± 1.7%; absolute range 1.4–39.4%). In contrast to sealed pots, where CO_2 could not disappear between the CO_2 trappings, it is reasonable that in open pots, rather constant CO_2 concentrations entailed a constant rate of $CaCO_3$ recrystallization. Therefore the complete time interval from the first labeling of A plants to the harvesting of C plants (182 days) was used for the calculation of recrystallization rates (Equation 4).

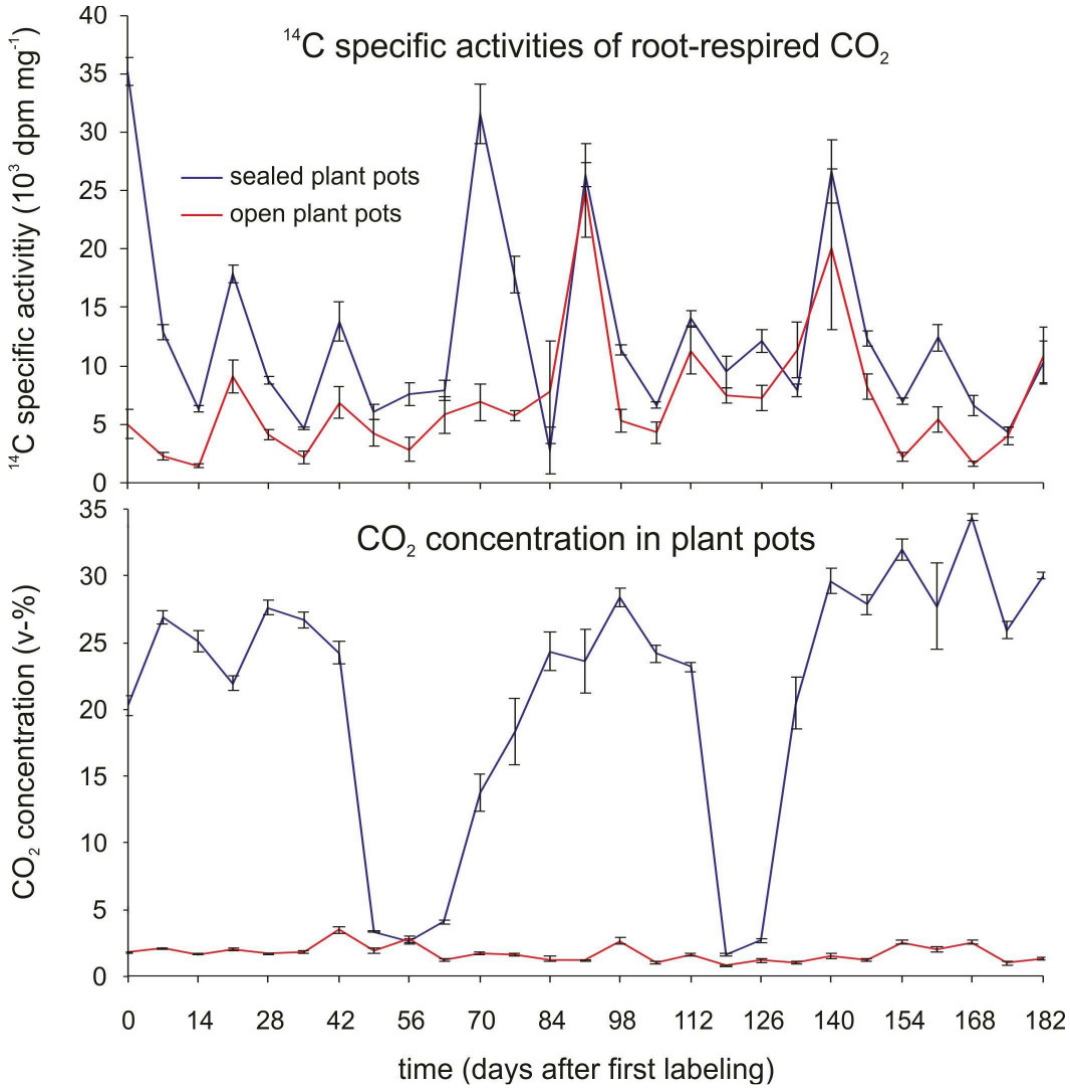

Figure 5-3. Temporal course of a) ^{14}C specific activities of root-respired CO_2 during the experiment, b) CO_2 concentrations in plant pots, accumulated during 7 days. Mean values ± SEM, n = 5.

The depth-dependent distribution of CO_2 concentrations in the columns was not determined in our experiment. However, we assume that depth-related variation of CO_2 concentrations in the plant pots was considerably lower than temporal variations shown in Figure 5-3b. It was shown previously that carbonate recrystallization rates increase with increasing CO_2 concentration, and that the relation is described by a saturation curve within the range of naturally occurring soil CO_2 concentrations (0.038 to 5%; *Gocke et al.* [2010a]).

In the present study, the mean value of 1.7% CO_2 was already near to saturation. Therefore, it was assumed that even in lower parts of the loess column, where amounts of root biomass were low, CO_2 concentrations were high enough, and did not limit $CaCO_3$ recrystallization.

3.4 Secondary $CaCO_3$

The total $CaCO_3$ content in each 5 cm-slice was identical before and after the experiment.

Based on ^{14}C labeling we calculated that 533.7 ± 28.2 mg of loess $CaCO_3$ (0.12 ± 0.01%) were recrystallized in the complete column (0–85 cm) after 182 days. This corresponds to an amount of secondary $CaCO_3$ of 368 g m^{-2}. The corresponding recrystallization rate amounts for $6.8 \cdot 10^{-6} \pm 0.4 \cdot 10^{-6}$ day^{-1}. Extrapolating this rate to longer periods, the time necessary for complete (95%) recrystallization of the loess carbonate was calculated. Likely, the carbonate (primary and secondary) is recrystallized several times before being incorporated in concretions. Therefore, an exponential approach was chosen for estimation of recrystallization periods (see chapter 2.4, Equation 5). Depending on length of the growing season (GS), periods between 14,400 years (GS one month) and 2,400 years (GS six months) were calculated for 95% recrystallization of total loess carbonate (Figure 5-4a).

Amounts of recrystallized $CaCO_3$ showed a depth-dependant distribution. However, largest amounts of recrystallized $CaCO_3$ (> 0.3% of total loess $CaCO_3$) did not occur in the depth of largest root biomass, but between 20 and 35 cm depth. Lowest amounts of recrystallized $CaCO_3$ (< 0.1%) were found in the uppermost 10 cm as well as in a depth of 45–85 cm (Figure 5-2b). This distribution results from $CaCO_3$ migration and should be considered when estimating recrystallization periods of loess carbonate.

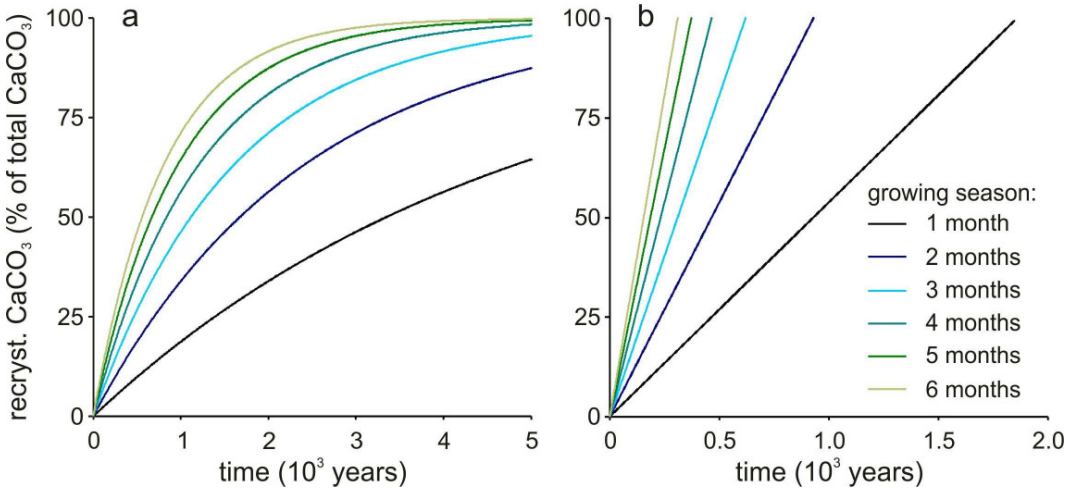

Figure 5-4. a) Recrystallization periods, based on recrystallization rate from the complete loess column: $6.8 \pm 0.4 \cdot 10^{-6}$ day^{-1}, b) Periods necessary for complete decalcification of the uppermost 15 cm of the loess column, based on theoretical rate calculated for this depth interval: $1.77 \pm 0.26 \cdot 10^{-5}$ day^{-1}. Mean values ± SEM, n = 5.

4 Discussion

4.1 ^{14}C distribution among C pools and methodological approach

The ^{14}C analyzed compartments (Table 5-1) accounted for 37.8 ± 2.0% of assimilated ^{14}C, indicating that the maize plants respired > 60% of the assimilated ^{14}C via the shoots. While in short-term ^{14}C labeling experiments (< 1 day) usually ^{14}C recovery rates of 60–70% are obtained (e.g. *Fischer et al.* [2010]), shoot respiration causes larger loss of input ^{14}C in experiments of 1 month and longer [*Werth and Kuzyakov*, 2006; this study].

The largest portion of assimilated ^{14}C was found in shoots, followed by roots (74.4 and 18.2%, respectively). Values for shoots were in a similar range like previous studies with maize, whereas root values were higher than literature data [*Werth and Kuzyakov*, 2006; *Nguyen et al.*, 1999]. This is related to the considerably longer duration of the present study when compared to other ^{14}C labeling experiments, thus allowing a larger portion of assimilated ^{14}C to be incorporated into root tissue. In contrast, during short-term experiments, a larger part of assimilates allocated belowground is used by grasses for root exudation and respiration [*Hill et al.*, 2007]. Except for root-respired CO_2, which did not represent true values in open pots because of CO_2 exchange with atmospheric air, loess $CaCO_3$ yielded the smallest portion of recovered ^{14}C activity. The reason for this is the faster accumulation of assimilates mainly in organic C pools (fresh biomass, rhizodeposits). Nevertheless, the calculation of very small amounts of recrystallized $CaCO_3$ was possible based on isotopic exchange between root-respired CO_2 and loess $CaCO_3$ after the ^{14}C labeling.

4.2 Depth-related distribution and accumulation rate of secondary $CaCO_3$

The total $CaCO_3$ content in each 5 cm-layer was similar before and after plant growth. This suggests that in time periods as short as in our experiment, possible mass changes resulting from redistribution of loess $CaCO_3$ by recrystallization and downward movement were too low to be detected by the method applied. In contrast to the total $CaCO_3$, the amounts of recrystallized $CaCO_3$ calculated based on incorporated ^{14}C, showed a depth-related distribution pattern with largest values in 20–35 cm (Figure 5-2b). In contrast, very small amounts of recrystallized $CaCO_3$ were found in the uppermost 5–10 cm and in the lower part of the loess column (45–85 cm). This pattern did not reflect the distribution of root biomass, but more resembled the graph of the final loess moisture with a defined peak in a certain depth (Figure 5-2a), indicating an effect of water percolation and alternating wetting and drying on the depth of carbonate accumulation. This suggests that in the uppermost part of the loess column (at least up to 15 cm depth) the $CaCO_3$ was dissolved and removed, whereas immediately below that depth (particularly 15–40 cm) the dissolved Ca^{2+} and $H^{14}CO_3^-$ were reprecipitated as $Ca^{14}CO_3$.

Under field conditions, main factors leading to this reprecipitation by driving the reaction of Equation 6 to the left, are decrease of CO_2 partial pressure, increase of the concentration of Ca^{2+} and HCO_3^-, or a combination of these [*Krauskopf and Bird*, 1995; *Birkeland*, 1999].

$$CaCO_3 + CO_2 + H_2O \leftrightarrow Ca^{2+} + 2\ HCO_3^- \quad (6)$$

Two explanations for the depth-related distribution of secondary carbonate obtained from the loess columns come into question. First, it might be deduced from the distribution of root biomass with highest amounts in the uppermost 5 cm, that the CO_2 concentration is very high at the top and strongly decreases beneath, causing the above mentioned change from prevailing dissolution into mainly $CaCO_3$ reprecipitaition. This might be expected because CO_2 concentration strongly influences rates of carbonate recrystallization [*Gocke et al.*, 2010a]: In vicinity of roots, higher rates are obtained when compared to soil distant from roots [*Gocke et al.*, 2010b] because of steep decrease of CO_2 concentration from the root surface towards root-free soil [*Gollany et al.*, 1993; *Hinsinger et al.*, 2003]. However, the high amounts of root biomass in 0–5 cm are mainly the result of three cycles of plant growth, with crown and brace roots of maize providing high amounts of plant biomass but only little fine root hairs producing CO_2. Ignoring the uppermost 5 cm, particularly high amounts of secondary $CaCO_3$ (20–35 cm) occurred within the zone of relatively high amounts of root biomass of 0.7–1 g (5–45 cm; Figure 5-2a) and not below. Moreover, only small amounts of recrystallized $CaCO_3$ were found in greater depth although the loess column was completely rooted and probably high CO_2 respiration rates, related to youngest roots [*Larionova et al.*, 2006] prevailed also in the deeper parts of the loess column. This makes the CO_2 concentration as the only reason for the distribution pattern of secondary carbonate rather unlikely.

Fine sandy and silty soil parent material, like e.g. loess, provides suitable conditions for downward transport of dissolvable substances by water percolation [*Jenny*, 1994]. Therefore, a second explanation: attributing the distribution of recrystallized $CaCO_3$ to downward transport of dissolved Ca^{2+} and HCO_3^-, is more likely. We hypothesize that downward transport of dissolved Ca^{2+} and $H^{14}CO_3^-$ did not take place directly by percolating water after watering of the maize plants, because this movement is too fast (usually between 10 and 100 cm day^{-1} in loessic soils) to dissolve and transport primary loess $CaCO_3$. Rather, the present solution (30% of WHC), saturated with respect to Ca^{2+} and CO_3^-, was displaced downwards by the next portion of water when the moisture level was again increased to 70% of WHC. However, reprecipitation of these ions as $CaCO_3$ due to increasing saturation of the soil solution results from a mixed effect of water regime (decreasing amounts of percolating water with increasing depth; *Arkley* [1963]) and roots (increasing water uptake; *Schlesinger* [1985]). This agrees with the observation that maximum amounts of recrystallized $CaCO_3$ occurred above the depth of maximum water content at 40–45 cm (this study; Figure 5-2a, b). The occurrence of minor amounts of secondary $CaCO_3$ also in depths below the zone of maximum loess moisture result from a small effect of roots which grew down to the bottom.

Based on previous findings [*Gocke et al.*, 2010a, 2010b], we assume that the amount of dissolved primary $CaCO_3$ in a certain depth depends mainly on soil CO_2 concentration and thus on the amount of living root biomass because in C_{org}-poor loess, CO_2 respired in the rhizosphere was the main source for C isotopic exchange with primary $CaCO_3$ by formation of secondary carbonate. Consequently, we calculated the amounts of $CaCO_3$ which should have been recrystallized theoretically in each 5 cm-slice ($\text{theoretically recryst.}\,CaCO_3\,(x)$) in

the case that no downward migration had occurred, i.e. assuming that each $CaCO_3$ molecule was dissolved and reprecipitated in the same depth.

$$\text{theoretically recryst.}\,CaCO_3\,(x) = \frac{\text{total measured recryst.}\,CaCO_3}{\text{total root biomass}} \bullet \text{root biomass}_x \qquad (7)$$

With $\text{total measured recryst.}\,CaCO_3$ as the amount of recrystallized carbonate within the complete loess column, calculated based on total ^{14}C activity in loess $CaCO_3$, $\text{total root biomass}$ as the total amount of biomass within the loess column and root biomass_x as the amount of root biomass in the according 5 cm-layer.

By subtracting the theoretically calculated amount from the amount measured based on ^{14}C activity in individual layers, the amount of allocated $CaCO_3$ ($\Delta(x)$) was determined for each depth segment:

$$\Delta(x) = \text{measured recryst.}\,CaCO_3 - \text{theoretically recryst.}\,CaCO_3 \qquad (8)$$

Negative values, suggesting leaching and thus reduction of the amount of secondary $CaCO_3$, were obtained for the uppermost 15 cm (Figure 5-2c). In this part of the loess column 81.5 ± 3.2% of the $CaCO_3$ which underwent dissolution and isotopic exchange with respired $^{14}CO_2$ was lost by downward migration. Probably this amount was overestimated because of very high amounts of root biomass but at the same time few fine root hairs in the uppermost 5 cm (as discussed above) and therefore, provides only a rough approximation. In contrast, positive values were calculated in a depth of 15–50 cm (Figure 5-2c). This zone comprised 72.6 ± 4.5% of the secondary $CaCO_3$ found in the loess column in total. Additionally to $CaCO_3$ recrystallized *in situ* in this depth, another 97.2 ± 8.5% of secondary $CaCO_3$ were previously eluted from upper layer and accumulated in this depth by reprecipitation. Referred to area, the secondary carbonate content in this accumulation zone (15–50 cm) was 284 g m^{-2}.

As high soil CO_2 concentrations are maintained mainly during the growing season [*Fierer et al.*, 2005], and permanent CO_2 supply by root and rhizomicrobial respiration impedes equilibrium of the system [*Gocke et al.*, 2010b], notable $CaCO_3$ recrystallization takes place predominantly during that time [*Kuzyakov et al.*, 2006]. The length of the growing season depends on climatic conditions and can be as short as 2 months in arid regions. As pedogenic carbonates are formed mainly in those regions, our experiment simulated 3 years, each of them with 2 months of growing season. In this case, the amount of accumulated $CaCO_3$ corresponds to a storage rate of 94 g m^{-2} yr^{-1}.

For natural soil profiles secondary carbonate contents were calculated based on parameters like densities, weights per unit area and thickness of the sampled interval [*Machette*, 1985]. The author obtained storage rates indirectly by dividing the carbonate content by the age of the soil, which was determined by other methods (e.g. based on archeological data or by numerical dating). Depending on climate, texture of soil parent material and carbonate buildup stage (as established by *Gile et al.* [1966]), different contents of pedogenic carbonate in calcic soils and paleosols were estimated, e.g. 22 kg m^{-2} [*Gile*, 1995], ~ 100 kg m^{-2} [*Gile*, 1995; *Landi et al.*, 2003] up to several 100 kg m^{-2} [*Sobecki and Wilding*, 1983; *Nordt et al.*, 2000]. Even under the arid climatic conditions of Lanzarote,

values for the carbonate storage can vary between 1 and 1,000 kg m^{-2} [*Scheffer and Schachtschabel*, 2002]. Carbonate storage rates in literature range mainly between 1 and 5 g $CaCO_3$ m^{-2} yr^{-1} for desert soils [*Schlesinger*, 1985; *Marion*, 1989], whereas somewhat higher rates of up to 14 g m^{-2} yr^{-1} were obtained in grassland and forest soils of semiarid regions in Canada [*Landi et al.*, 2003]. The main reason for the considerably higher rate obtained in the present study (94 g m^{-2} yr^{-1}) is that most of the cited literature data were obtained from soils developed on non-calcareous parent material or parent material containing carbonate solely as dolomite. Hence, rates of carbonate accumulation in these soils are limited by the rate of Ca^{2+} influx by dust and / or rainfall as well as the amount of Ca^{2+} entering the soil instead of being washed out. In contrast, for calcareous parent materials like loess, not Ca^{2+} availability, but rates of $CaCO_3$ dissolution and translocation by leaching waters are limiting factors for secondary carbonate accumulation [*Arkley*, 1963; *Birkeland*, 1999]. Further factors leading to the high accumulation rate of 94 g $CaCO_3$ m^{-2} yr^{-1} are 1) CO_2 concentration in the plant pots (1.7%) near to the upper limit of naturally occurring values [*Brook et al.*, 1983], 2) humid conditions during the experiment, both of these promoting dissolution of primary loess $CaCO_3$, 3) strongly contrasting moisture conditions with faster alternation between 'humid' and 'arid' conditions than under natural conditions.

4.3 Time needed for complete leaching of $CaCO_3$ from upper horizons

Because secondary carbonate accumulation did not occur in the uppermost part of the loess column, we suggest that the rate of carbonate migration, resulting from downward transport of dissolved Ca^{2+} and HCO_3^- by percolating water, is higher than the rate of *in situ* recrystallization of loess $CaCO_3$ by C exchange between carbonate and root-respired CO_2. It was calculated for very old soils of Pleistocene age that strong leaching resulted in loss of both pedogenic and lithogenic carbonate [*Nordt et al.*, 2000]. However, we assume that in our experiment only secondary carbonate was transported downward, i.e. isotopic exchange of primary $CaCO_3$-C and ^{14}C from root and rhizomicrobial respiration occurred prior to or simultaneously with carbonate migration. This can be expected because only dissolved Ca^{2+} and HCO_3^- can be transported by percolating water and isotopic equilibrium between dissolved carbonate and respired CO_2 is reached within few days [*Gocke et al.*, 2010a]. This indicates that, given the permanent presence of moisture, the rate at which a carbonate accumulation horizon forms in loess is limited by the $CaCO_3$ recrystallization rate rather than the rate of its downward transport. This possibility was mentioned before by *Arkley* [1963]. Hence, recrystallization rates based on the theoretical amounts of secondary carbonate (Equation 7) were calculated for the uppermost 15 cm where secondary $CaCO_3$ was lost due to downward migration. The rate was $1.77 \cdot 10^{-5} \pm 0.26 \cdot 10^{-5}$ day^{-1}. As the uppermost 15 cm of the loess column were dominated by $CaCO_3$ depletion (Figure 5-2c), only the first part of the recrystallization process, i.e. $CaCO_3$ dissolution, is relevant here. Each $CaCO_3$ molecule is dissolved once before being transported to deeper layers. Therefore, to calculate time periods necessary for decalcification of the uppermost 15 cm we did not use the exponential approach shown in chapters 2.4 and 3.4, but assumed a linear removal of primary $CaCO_3$. Thus, dissolution of 95% of total loess carbonate in the uppermost 15 cm takes between 300 years

(GS six months) and 1,760 years (GS one month; Figure 5-4b). This implies that for loess containing 27.4% primary $CaCO_3$, at least hundreds of years are necessary for leaching of upper loess layers and carbonate accumulation in deeper horizons by pedogenic processes at precipitation of 18.5 mm day^{-1}.

4.4 Comparison with field conditions

Under controlled conditions of our study, the calculated time intervals of 300–1,760 years have to be regarded as maximum ages for carbonate accumulation horizons in loess. Under field conditions, however, periods in which significant amounts of pedogenic carbonate are accumulated are longer. This study underestimated the time intervals because of

- high CO_2 concentrations in plant tubes (1.7%) favoring dissolution of primary loess $CaCO_3$,
- high precipitation (18.5 mm day^{-1}) enforcing dissolution of $CaCO_3$ and fast downward transport of dissolved Ca^{2+} and HCO_3^- and
- overestimated theoretical amounts of recrystallized carbonate and corresponding recrystallization rates in the uppermost 15 cm of the loess column because of high amounts of root biomass in the uppermost 5 cm.

Further it has to be considered that

- within the accumulation horizon, $CaCO_3$ is dissolved and reprecipitated several times before forming concretions or an impregnated horizon,
- during pedogenesis, the carbonate accumulation zone might migrate downward, usually below the depth of maximal soil moisture [*Birkeland*, 1999] which was not yet the case after six months (Figure 5-2), and
- in regions of arid climate, grass vegetation with less root biomass compared to maize might lead to lower recrystallization rates, resulting in longer recrystallization periods.

Various time intervals for leaching and carbonate accumulation between 300 years and more than 10,000 years were obtained or suggested from natural soil and paleosol profiles by different methodological approaches: Under humid climates of Northwestern Europe, 300 years were sufficient for complete leaching of calcareous parent materials with less than 10% initial $CaCO_3$ content [*Jenny*, 1994]. In contrast, *Arkley* [1963], on a theoretical basis of depth-dependent water movement, calculated typical ages of carbonate accumulation horizons in loess soils of 8,500 – 11,600 years for the semiarid Great Plains. For soil development on non-calcareous parent material, *Lal and Kimble* [2000] assumed that probably tens of thousands of years are necessary for the accumulation of carbonate amounts sufficient to form calcic horizons which can be attributed to the final stage VI of carbonate accumulation according to *Machette* [1985]. This suggests that the accumulation of significant amounts of secondary carbonate in Holocene soils is possible and that carbonate content of the parent material effects its rate. Another important factor for the duration of carbonate accumulation is precipitation. Considering the fact that pedogenic carbonates accumulate mainly under arid to semiarid climatic conditions, our study probably underestimated the time necessary for formation of calcic horizons, because dry conditions lead to lower carbonate recrystallization

rates. However, under more humid climatic conditions, shorter periods than the above mentioned are required for formation of calcic horizons: *Alexandrovskiy* [2000] showed for the Northern Caucasus region that a carbonate accumulation horizon had developed below an anthropogenic limestone pavement on a loessic funeral mound during the second half of the Holocene, within only 3.5 – 5 kyrs. In terms of magnitude, these data fit with our modeled accumulation periods based on the ^{14}C approach.

5 Conclusions

The ^{14}C labeling and isotopic exchange approach was applied to estimate amounts of secondary $CaCO_3$ by quantifying ^{14}C, assimilated by plants and respired in rhizosphere. Carbonate recrystallization rates in the range of 10^{-5} to 10^{-6} day^{-1} were obtained. The secondary carbonate showed a distinct depth-related distribution with highest amounts in the zone between 20 and 35 cm, i.e. within a zone of high amounts of root biomass, but above the zone of maximal loess moisture measured at the end of the experiment. Presumably, the depth interval of maximal accumulation of secondary $CaCO_3$ was determined by decreasing amounts of percolating water with increasing depth, as well as by water uptake by roots, both leading to increasing saturation of the soil solution with respect to Ca^{2+} and HCO_3^-, and finally reprecipitation of $CaCO_3$. Assuming migration of secondary carbonate and the dependence of *in situ* dissolved carbonate amounts on amounts of root biomass, we showed that more than 80% of recrystallized $CaCO_3$ were removed from the uppermost 15 cm of the loess column within six months of plant growth. Based on calculated recrystallization rates and depending on the length of growing season, periods of at least 300, but probably more than thousand years are necessary for complete decalcification of the uppermost 15 cm. Under the rather humid experimental conditions, these periods represent the maximum time of decalcification of upper soil horizons and formation of carbonate accumulation horizons.

Based on redistribution of ^{14}C in carbonate we clearly showed that a calcic horizon is formed by migration of recrystallized $CaCO_3$ and so, its isotopic signature can be used for paleoenvironmental reconstruction. However, the precision of such reconstructions cannot be better than the periods necessary for calcic horizon formation.

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Study 6: Carbonate rhizoliths in loess and their implications for paleoenvironmental reconstruction revealed by isotopic composition: $\delta^{13}C$, ^{14}C

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Abstract

Loess-paleosol sequences are important terrestrial archives for studying Quaternary climate changes. They often contain secondary carbonates including e.g. rhizoliths (calcified roots). These secondary carbonates are precipitated in isotopic equilibrium with root-derived CO_2 and are therefore used to reconstruct the vegetation present during their formation based on stable carbon isotopic composition ($\delta^{13}C$). Usually, the chronological context of secondary carbonates in general is not mentioned. Rhizoliths are assumed to have formed synsedimentarily with loess deposition.

The loess-paleosol sequence at Nussloch, SW Germany, contains in its youngest part (Upper Würmian) large carbonate rhizoliths with diameters of up to 5 cm and lengths of up to 1 m and more, which have not been described in this profile so far. We investigated rhizoliths as well as loess adjacent to and distant from rhizoliths for carbonatic carbon (C_{carb}) and organic carbon (C_{org}), as well as carbon isotopic analyses ($\delta^{13}C$, radiocarbon dating), for identification of rhizolith formation and carbonate dynamics in loess sequences. Considering the ^{13}C fractionation by carbonate precipitation, the $\delta^{13}C$ of the rhizolith carbonate (-10.9 ± 0.1‰) revealed their C_3 plant origin and the absence of large amounts of occluded primary loess carbonate. Similar ^{14}C ages of C_{carb} and C_{org} in rhizoliths (3788 ± 59 years B.P. and 3150 ± 59 years B.P., respectively) showed that they were not altered post-segregationally and therefore are suitable for paleoenvironmental reconstructions. These ages clearly show that rhizoliths did not form synsedimentary. Roots entered the sediment later, after the loess deposition had ceased at ~ 15 ka B.P. at Nussloch. Even in the loess adjacent to the rhizoliths (up to a distance of 5 cm), $\delta^{13}C_{carb}$ values indicate the presence of secondary carbonate deriving from post-sedimentary incorporated organic matter of other origin than that of the reference loess material. Hence, this post-sedimentary input of younger root biomass might have masked the initial plant signal in loess-paleosol sequences, which could cause uncertainties for paleoenvironmental reconstructions based on loess organic matter.

Keywords: Calcified roots / loess / radiocarbon age / $\delta^{13}C$ / secondary carbonate / micromorphology

1 Introduction

Loess-paleosol sequences represent one of the most informative terrestrial paleoclimatic archives (Pye and Sherwin, 1999). A broader application of dating techniques to loess-paleosol sequences, especially optically stimulated luminescence (OSL) method (reviewed by Roberts, 2008; Kadereit et al., 2010), as well as detailed sedimentological (Kemp et al., 1994; Xiao et al., 1995) and paleopedological studies (Mason et al., 2008) contributed to an improved understanding of past climate fingerprints in loess and corresponding terrestrial environments. In those pedosedimentological complexes, secondary carbonate accumulation occurs frequently among the most prominent pedogenic features. Together with loess dolls, remains of calcified roots are a typical feature of loess-paleosol sequences (Becze-Deák et al., 1997; Kosir, 2004). Cerling (1984) showed for the first time that secondary carbonate in

modern soils forms in isotopic equilibrium with soil CO_2, which derives mainly from root respiration and microbial decomposition of soil organic matter (Deines, 1980). Secondary carbonates are therefore used as an important tool for the reconstruction of paleoenvironmental conditions by stable carbon and oxygen isotopic composition ($\delta^{13}C$, $\delta^{18}O$; Quade and Cerling, 1995; Mora et al., 1996; Buck and Monger, 1999; Pustovoytov et al., 2007a), as well as for radiocarbon dating (^{14}C) of soils and paleosols (Amundson et al., 1994; Pustovoytov et al., 2007b). One major prerequisite is that incorporation of older C (e.g. from parent material) and younger C (by post-segregational alteration) can be excluded. In loess deposits, secondary carbonates have been studied by a series of instrumental methods, including micromorphology (Klappa, 1980; Becze-Deák et al., 1997; Kosir, 2004; Wang et al., 2004; Wang and Greenberg, 2007), stable carbon isotope composition ($\delta^{13}C$; Wang et al., 2000; Pustovoytov and Terhorst, 2004; Wang and Greenberg, 2007; Łącka et al., 2009) and radiocarbon dating (Pustovoytov and Terhorst, 2004; Asano et al., 2007).

Most studies based on carbon isotopic composition ($\delta^{13}C$, ^{14}C) of secondary carbonates were performed on carbonate coatings on clasts (Amundson et al., 1989; Monger et al., 1998; Pustovoytov et al., 2007a, b) and small secondary carbonate concretions including soft powdery lime, pseudomycel or small nodules (Buck and Monger, 1999; Khokhlova et al., 2001). Becze-Deák et al. (1997) recognized the high potential of calcified roots for chronological and paleoenvironmental studies due to their formation in rather short periods. For instance, Jaillard (1992) observed that crystals of calcified root cells can form within 24 hours. Calcified roots are termed in literature under different names: beinbrech, osteokollen (Greek osteon = bone, Latin collon = stem; Ziehen, 1980), rhizomorphs, rhizo(con)cretions, root casts, pedotubules, rhizoliths (summarized by Klappa, 1980) from which we chose the term ‘rhizolith’. Rhizoliths are formed by encrustation of plant roots by secondary carbonate (Klappa, 1980; Jaillard et al., 1991; Becze-Deák et al., 1997). They occur in sandy and silty calcareous sediments (Ziehen, 1980; Becze-Deák et al., 1997; Cramer and Hawkins, 2009).

Although calcified root cells and small rhizoliths were used for several studies as a tool for reconstructing paleoenvironmental conditions (Wang et al., 2000; 2004) and paleovegetation (Wang and Greenberg, 2007), the chronological context of these calcified roots was never determined by radiocarbon dating in these studies. Instead, the authors linked the age of the rhizoliths to the age of surrounding loess or paleosol organic matter (OM). However, as shown by Pustovoytov and Terhorst (2004) for calcified root cells from SW Germany, the ages of loess and secondary carbonate nodules may not necessarily be the same, rather the secondary carbonates may have formed after loess sedimentation by encrustation of plant roots penetrating the loess-paleosol sequence after sedimentation. Therefore, the abovementioned studies based on stable carbon isotopic composition of secondary carbonate in loess-paleosol sequences should be regarded with caution. Two further factors complicate the correct interpretation of paleoenvironmental and chronological information within secondary carbonates as e.g. rhizoliths: On the one hand, incorporation of older carbonate, e.g. from parent material into the secondary carbonates might entail an overestimation of ^{14}C ages. On the other hand, post-segregational alteration of the secondary carbonate,

introducing younger atmospheric CO_2, can distort the chronological information used for radiocarbon dating and lead to an underestimation of radiocarbon ages of secondary carbonate (Chen and Polach, 1986; Amundson et al., 1994; Budd et al., 2002).

Most studies about secondary carbonates in loess-paleosol sequences focused on relatively small-sized calcified roots (some 0.5–2 mm in diameter and 5–20 mm in length). Beyond this morphological variety, there are also relatively large, solid calcified roots (diameter up to 5 cm, length up to 1 m and more), which, however, occur less frequently in loess-paleosol sequences (Ziehen, 1980) and were mentioned in SW Germany only from calcareous dune sands (Löscher and Haag, 1989). Presumably, such large rhizoliths are suitable to improve our knowledge about the chronological context of this special form of secondary carbonate and the potential contamination with older or modern carbon. Therefore, we investigated large calcified roots obtained from the late Quaternary loess-paleosol sequence at Nussloch (SW Germany) to elucidate their potential for paleoenvironmental research and loess chrono-stratigraphy.

Better understanding of the origin and formation of rhizoliths in loess-paleosol sequences, and thus of carbonate dynamics in calcareous sediments in general, can help to improve interpretations of paleoenvironmental and chronological data in loess-paleosol sequences. For our study, we chose rhizoliths from Nussloch, SW Germany, because this loess-paleosol sequence is properly investigated (Rousseau et al., 2007; Antoine et al., 2009) and provides a well developed Weichselian loess interval of up to 18.5 m thickness (Antoine et al., 2001). The rhizoliths are well accessible in a fresh section without alteration by modern pedological processes. We wanted to determine the portions of primary and secondary carbonate in rhizoliths and loess, as well as potential incorporation of OM from former root biomass in loess distant to rhizoliths. Therefore, we analyzed C_{carb} and C_{org} contents and their isotopic composition in microtransects through rhizoliths (from the center to the outer parts) as well as in macrotransects from rhizoliths via rhizosphere loess towards reference loess, which was obtained from the same depth as rhizoliths, but at a distance of 50–70 cm from rhizoliths. Micromorphological investigation of rhizoliths were used to support and improve understanding of results from carbon (C) analyses. The aim of this first study on inorganic and organic C in rhizoliths and surrounding materials was to prove their relevance for paleoenvironmental studies of terrestrial sedimentary sequences.

2 Methods

2.1 Study site

The study site is located near Nussloch, SW Germany, on the eastern side of the Rhine Rift Valley, about 10 km south of Heidelberg. Loess and rhizoliths were sampled at the open cast mine of HeidelbergCement AG (49.19°N, 8.43°E, 217 m NN). The loess-paleosol sequence with a total thickness of 18.5 m was formed during the last glacial-interglacial cycle (Zöller et al., 1988; Hatté et al., 1998) and shows the climatic record of the last 130 ka (Hatté

et al., 1999). A detailed description of the Nussloch sequence is provided by Antoine et al. (2001).

Contrary to previous descriptions of the Nussloch loess profile (Antoine et al., 2001, 2009), rhizoliths with irregular shape and size occur in a recently prepared section nearby (~ 100 m) the standard profile P4 (Antoine et al., 2001) from 1 m below the present surface down to a depth of at least 8 m. The rhizoliths reach sizes of up to 5 cm in diameter (Fig. 6-1) and can be traced over depth intervals of several dm up to ca. 1.5 m. In contrast to rhizoliths, large loess dolls, the formation of which does not occur in close spatial relation to roots, occur from 6 m downwards.

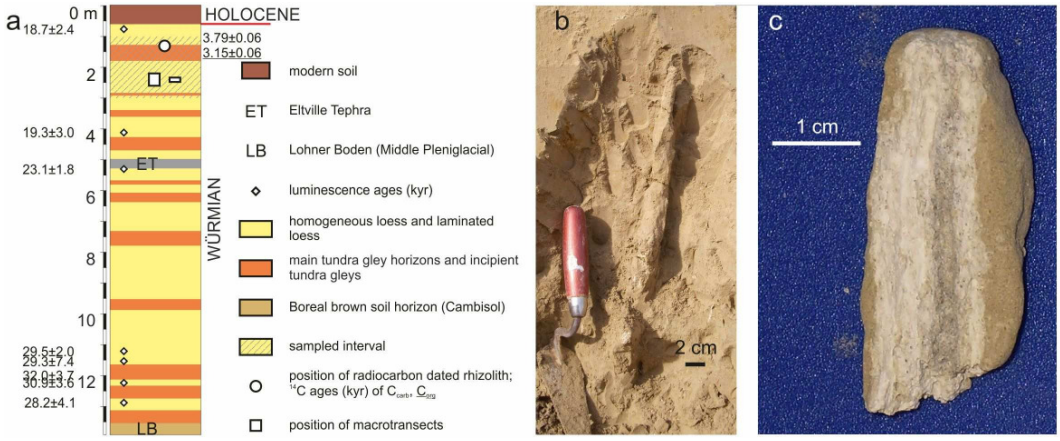

Fig. 6-1. (a) Stratigraphical chart of the Würmian Upper Pleniglacial loess sequence at Nussloch, modified after Antoine et al. (2009). For luminescence data see Antoine et al. (2009) and references therein. (b) Rhizolith *in situ*. (c) Longitudinal cut through a rhizolith.

2.2 Sampling

Würmian typical loess was sampled from a depth of 15 m below present surface (in the following named 'reference loess 1') to ensure that the loess is completely primary and not affected by current soil forming processes, i.e. recrystallization of loess carbonate or input and decomposition of fresh plant biomass. Rhizoliths of different shape and diameter were sampled from a depth interval between 1 m and 3 m below present surface. Correlation with the standard profile P4, described by Antoine et al. (2009), ensured that no paleosols were sampled. Due to limited amounts of available material for each rhizolith (especially for $\delta^{13}C_{org}$ determinations and studies of internal transects), different analyses (see below) were performed on different rhizolith samples. As the rhizoliths were most abundant between 1 m and 3 m and reached lengths of more than 1 m, differences related to sampling depth were not relevant.

For two rhizoliths from a depth between 2.2 m and 2.6 m below present surface, surrounding loess was sampled in *macrotransects* from the surface of the rhizolith up to a distance of 2.5 cm (± 0.5 cm; 'rhizosphere loess 1'), and 2.5–5 cm (± 0.5 cm; 'rhizosphere loess 2'). '). Herefore, rhizoliths with adhering loess material were collected and the latter was peeled off by a knife. One typical loess sample without visible root remains was taken from the same depth like these rhizoliths in a distance of 50–70 cm from rhizoliths (in the following named 'reference loess 2'). This sample set refers to Rhizolith 1 and Rhizolith 2 in

Fig. 6-3. Sizes of the samples depended on the sample type and, in case of rhizoliths, on diameter and length: Amounts were 20–100 g for individual rhizolith samples, > 50 g for rhizosphere loess samples, and > 300 g for reference loess samples. Dried samples were milled in a ball mill and homogenized. Note that reference loess 2, covering a depth of 40 cm, encompasses sediment accumulated during several thousand years and thus possibly represents a mixture regarding e.g. OM composition and further properties. However, the aim of this study was the comparison of rhizolith-derived OM with synsedimentary loess OM in general and not related to individual stratigraphic layers within the profile.

From a depth between 1 and 3 m, three rhizoliths with diameters of 7, 11 and 16 mm (Rhizoliths 3–5 in Fig. 6-5) were cut longitudinally and carbonate material was abraded by a scalpel in several *microtransects*, each of these with 4–5 distinct samples from the middle of the carbonatic tube to its outer boundary (Fig. 6-1c). Carbonate material of the microtansects was used for $\delta^{13}C_{carb}$ analyses.

Additionally, one loess doll was sampled at a depth of 6 m for comparison with rhizoliths.

After sampling, samples were immediately oven-dried at 60 °C until constant weight and stored dry thereafter.

2.3 Micromorphology

Four air dried rhizoliths were impregnated with Oldopal P80-21, cut and polished to 4.8 cm · 2.8 cm slices following the procedure of Beckmann (1997). Thin sections were described under a polarising microscope (Zeiss Imager.A2; Software AxioVision 4.7.2) mainly using the terminology of Stoops (2003). Oblique incident light (OIL) was obtained by an external light source with a double-arm swan neckglass fibre light guide.

2.4 Elemental analyses

Total C_{carb} content of the rhizoliths was determined gravimetrically from ten samples which were taken from depths of 1.1 m, 1.3 m, and between 1.5 and 2 m. The washed and dried (60 °C) samples were crushed, weighed, reacted with 1 M HCl in excess, and the residuum was subsequently weighed again. Two further rhizoliths and reference loess samples were analyzed for C_{org} and C_{carb} contents by combustion in an oven (Feststoffmodul 1300, Analytikjena) at 550 °C and 1000 °C, respectively. Results of elemental analyses are expressed in mg g^{-1}, with a relative precision of 1%.

2.5 $\delta^{13}C$ analysis

$\delta^{13}C$ analysis was performed on loess C_{carb} (6 samples) and loess C_{org} (5 samples), as well as on secondary carbonate and OM of the rhizoliths. In total, 25 microtransect samples and two bulk rhizolith samples were analyzed for $\delta^{13}C_{carb}$. Few of the rhizolith samples contained remains of ancient root biomass, presumably deriving from shrub or tree vegetation (Gocke et al., 2010a). $\delta^{13}C_{org}$ of rhizoliths was measured in 4 samples.

For $\delta^{13}C_{carb}$ analysis of loess or rhizoliths, aliquots of the samples were heated for 1 hour in a muffle furnace at 550 °C to remove organic matter. For $\delta^{13}C_{org}$ analysis, aliquots of the

samples were treated with 1 M HCl to remove $CaCO_3$, subsequently with few drops of 3 M HCl to remove less soluble carbonates like dolomite, and finally washed with de-ionized water. After adding water and shaking the samples, the solid material was sedimented by centrifugation and the supernatant was subsequently removed. After neutralization, samples were dried in an oven at 40 °C.

Stable carbon isotope analyses were performed at the Department of Food Chemistry, University of Hohenheim, Germany on a Delta Plus XL isotope ratio mass spectrometer (Thermo Finnigan MAT, Bremen, Germany) connected to an elemental analyzer EA 3000 (Hekatech, Wegberg, Germany). Results are given in ‰ calculated against V-PDB standard, with an absolute precision of < 0.5‰.

2.6 Calculation of secondary carbonate portions

Using $\delta^{13}C_{carb}$ values of the rhizolith and of loess parent material, the percentage of the carbon fraction originating from root and rhizomicrobial respiration (= portion of secondary carbonate) was calculated as follows (Nordt et al., 1998):

$$\%\ \text{secondary carbonate} = \frac{\delta^{13}C_{\text{rhizolith } C_{carb}} - \delta^{13}C_{\text{parent } C_{carb}}}{\delta^{13}C_{\text{secondary } C_{carb}} - \delta^{13}C_{\text{parent } C_{carb}}} \cdot 100 \qquad (1)$$

where $\delta^{13}C_{\text{rhizolith } C_{carb}}$ and $\delta^{13}C_{\text{parent } C_{carb}}$ are the isotopic signatures of the rhizolith carbonate and the primary loess carbonate (from reference loess 2). $\delta^{13}C_{\text{secondary } C_{carb}}$ is the isotopic signature of pure secondary carbonate (Nordt et al., 1996):

$$\delta^{13}C_{\text{secondary } C_{carb}} = \delta^{13}C_{vegetation} + \varepsilon + \nu \qquad (2)$$

where $\delta^{13}C_{vegetation}$ is the isotopic signature of the plant biomass leading to the formation of the secondary carbonate, ε is temperature dependant isotopic fractionation by carbonate equilibrium reactions (Romanek et al., 1992), and ν = 4.4‰ is the isotopic fractionation by molecular diffusion of CO_2 (O'Leary, 1981; Nordt et al., 1998). Assuming an average value for ε of 10.5‰, this results in secondary carbonate $\delta^{13}C$ signatures which are ~ 14.9‰ higher than those of the corresponding plant biomass.

Portions of secondary carbonate were calculated in the same way for the two macrotransect sample sets.

2.7 Radiocarbon dating

One rhizolith from a depth of 1.3 m below present surface was chosen for radiocarbon dating. The age of OM in reference loess was not determined as luminescence data for the Nussloch section are available from literature: Loess accumulation in this region ceased at 15 ka before present (Antoine et al., 2001). The loess surrounding the above mentioned rhizolith has an age of at least 17 ka, but is younger than 20 ka (age of the Eltville Tephra; Fig. 6-1a; Antoine et al., 2001).

^{14}C ages were determined in bulk rhizolith carbonate and in connected root biomass by the AMS facility at the Ångstrom Laboratory of the University of Uppsala, Sweden. The measured ages were converted to the Calendric Age BP using the online version of CalPal (www.calpal-online.de; quickcal2007 ver.1.5; Weninger et al., 2007) and are reported at the 2σ range and as mean value ± 1σ.

2.8 Calculations and statistics

For replicate analyses mean values and standard errors of the mean are presented.

Data sets were tested for significance of differences using one-way ANOVA with a significance level of 0.05, followed by post hoc Scheffé test. All statistics were done with STATISTICA 7.0 software.

3 Results

3.1 Micromorphology of rhizoliths

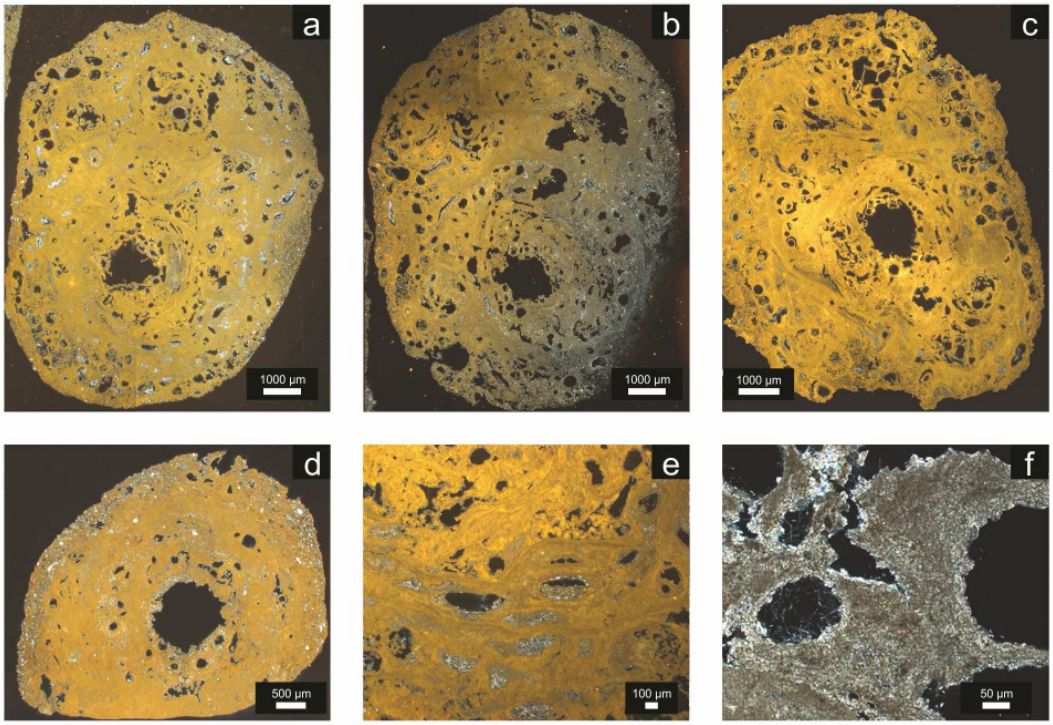

Fig. 6-2. Cross sections of four different rhizoliths with combined oblique incident light (OIL) and crossed polarizers (XPL); in OIL micrite appears yellowish. (a) whitish patches are sparite infillings; five centres of higher porosity are visible; total porosity is 20%. (b) four to five centres of higher porosity are visible; total porosity is 30%. (c) three centres of higher porosity are visible; whitish patches are sparite infillings; total porosity is 25%. (d) one central channel is surrounded by circular arranged smaller channels (diameter 50-100µm), total porosity is 15%. (e) magnification of (a), OIL+XPL: complete and incomplete sparite infillings, loose incomplete crumby infillings (lower left and right hand side), note the cloudy appearance of the micromass and the brighter colours of the more central parts of the rhizolith. (f) magnification of (c), XPL: needle fibre calcite in channels.

Matrix of the rhizolith carbonate occurs in all thin sections with an open porphyric c/f related structure. Micromass is mainly built up by micritic carbonate and coarse material

by sparitic carbonate. Other minerals like quartz, feldspars, and micas, which are attributed to primary loess material, occur only close to the outer boundary areas of the rhizoliths (Fig. 6-2a, d). A cloudy to circular striated appearance (in OIL) of the micromass shows heterogeneous parts. Cross sections show at least one channel (diameter around 1000µm) in the central part of the rhizoliths (Fig. 6-2a–d). As can be seen in longitudinal sections (Fig. 6-1c), this channel represents the former central cylinder of the root. In three rhizoliths (Fig. 6-2a–c) more than one center of higher porosity is detectable. Some of the channels have incomplete to complete sparite fillings, which is well expressed as whitish to greyish patches in Fig. 6-2a and 6-2c or loose crumbly incomplete fillings (2a-c, e). Rhizolith carbonate consisted mainly of micrite, whereas sparitic channel fillings in all rhizoliths, as well as needle fibre calcite that occurs in one rhizolith (Fig. 6-2f; Stoops, 1976) indicate the presence of post-segregationally recrystallized carbonate within rhizoliths.

3.2 C_{org} and C_{carb} content of rhizoliths and loess

According to Bente and Löscher (1987), the Nussloch loess-paleosol sequence shows average C_{carb} contents of 30 mg g^{-1} in loess and 36 mg g^{-1} in the Upper Würmian tundra gleys ('Nassböden'), corresponding to $CaCO_3$ contents of 25% and 30%, whereas C_{org} contents in general are < 5 mg g^{-1}.

Reference loess 1 contained 35 mg C_{carb} g^{-1} (29% $CaCO_3$), while it was 46 mg g^{-1} for reference loess 2 (38% $CaCO_3$). These rather high carbonate contents are in accordance with previously reported data from Nussloch (Hatté et al., 1998). The C_{carb} content of the rhizoliths varied between 88 and 116 mg g^{-1} (74–97% $CaCO_3$) and averaged at 102 ± 3 mg g^{-1} corresponding to 85 ± 2% $CaCO_3$ (Fig. 6-3a). Variations of C_{carb} contents between rhizoliths of different depth were minor and not significant. This average rhizolith C_{carb} content is considerably higher than that of a loess doll (80 mg g^{-1}) from the same profile sampled at a depth of 3.5 m. C_{carb} contents of rhizoliths were significantly ($p < 0.01$) higher than those of all loess samples (rhizoloess and reference loess).

C_{org} content was 0.3 mg g^{-1} for reference loess 1 (Wiesenberg et al., 2010) and 8.3 mg g^{-1} for reference loess 2. Rhizoliths showed considerably higher C_{org} contents which differed between the samples depending on the amount of root residues. For two samples from depths between 2.2 m and 2.6 m, the C_{org} content was 54.2 mg g^{-1} and 88.9 mg g^{-1} (Gocke et al., 2010a). C_{org} contents of rhizoliths were significantly ($p < 0.05$) higher than those of rhizoloess and reference loess.

C_{carb} and C_{org} contents of rhizosphere loess samples were similar to those values of reference loess 2, sampled at the same depth (Fig. 6-3a).

3.3 Stable carbon isotopic composition ($\delta^{13}C$)

$\delta^{13}C$ analyses yielded significantly ($p < 0.01$) different results for rhizolith C_{org} and for C_{carb} in rhizoliths, reference loess 1 and reference loess 2 (Fig. 6-4). The carbon isotopic signature of rhizolith C_{org} was –25.9 ± 0.5‰. Minimum and maximum $\delta^{13}C$ values of rhizolith C_{carb} were –12.0‰ and –10.1‰, with an average of –10.9 ± 0.1‰. The isotopic signature of primary loess C_{carb} from a depth of 15 m below present surface (reference loess

1) was –1.2 ± 0.1‰, whereas it was –2.4 ± 0.1‰ for root-free loess in a depth of 2.2–2.6 m (reference loess 2; Fig. 6-4). The latter value is in agreement with literature data from uppermost Würmian loess in the region south of Heidelberg (Pustovoytov and Terhorst, 2004).

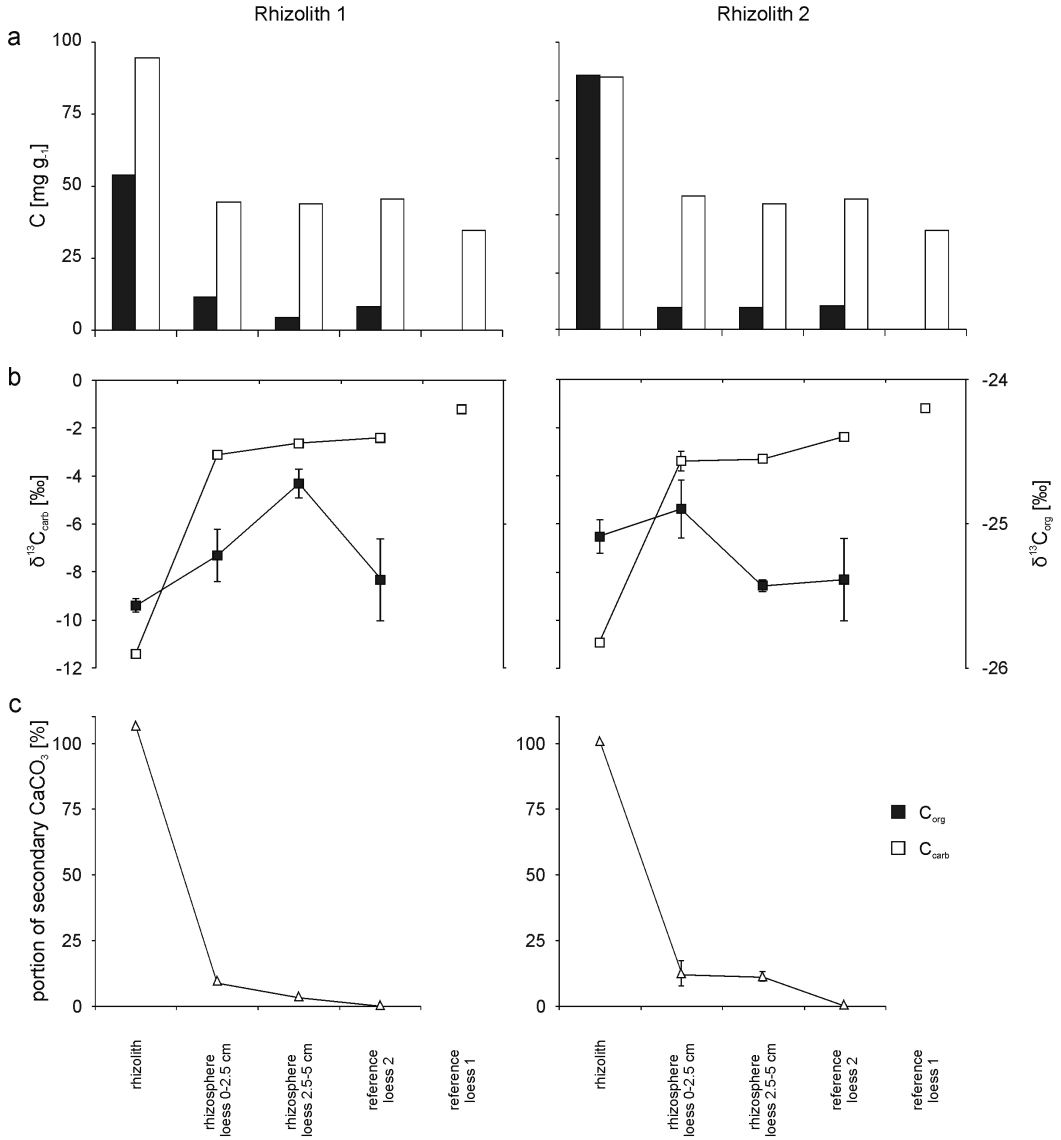

Fig. 6-3. Macrotransects of two rhizolith samples from a depth of 2.2 – 2.6 m comprising the rhizolith itself, loess adjacent to the rhizolith and reference loess 2. For comparison, results from reference loess 1 (depth 15 m) are also shown. (a) C_{org} and C_{carb} ($CaCO_3$) contents, (b) $\delta^{13}C$ values of C_{org} and C_{carb}, (c) portions of secondary carbonate in rhizolith and loess, assuming a $\delta^{13}C$ difference between $\delta^{13}C_{vegetation}$ and $\delta^{13}C_{pedogenic\ CaCO_3}$ of 14.9‰. Error bars (partly smaller than symbol size) show standard error of the mean (SEM) between replicates.

Within the *macrotransects*, $\delta^{13}C_{carb}$ values in rhizoliths were around –11‰ and showed increasing values towards rhizosphere loess as well as from rhizosphere loess towards reference loess 2. $\delta^{13}C_{carb}$ values were significantly different from each other ($p < 0.05$) except for rhizoloess 2, which was not different from rhizoloess 1 and reference loess 2. $\delta^{13}C_{org}$

values within the macrotransects did not show any clear trend. They varied between –24.7 and –25.8‰ without significant differences (Fig. 6-3b).

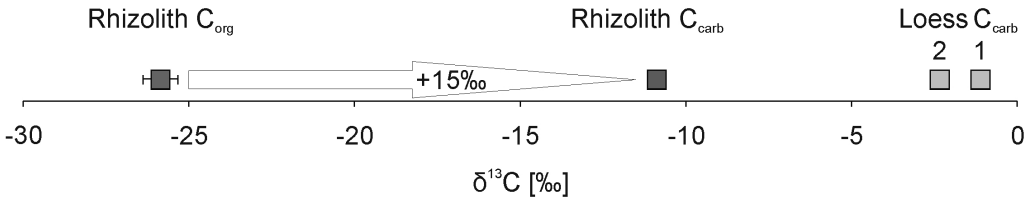

Fig. 6-4. Average $\delta^{13}C_{carb}$ values from loess (reference loess 1 from 15 m below present surface, reference loess 2 from 2.2–2.6 m below present surface) and rhizoliths, and $\delta^{13}C_{org}$ values from rhizoliths. Error bars (partly smaller than symbol size) show SEM between replicates.

$\delta^{13}C_{carb}$ *microtransects* of the smallest (rhizolith 3) and the medium size (rhizolith 4) rhizolith samples showed slightly increasing values from the center to the margin of the tube from –11.2 to –10.6‰ and from –11.1 to –10.6‰, respectively. For the rhizolith sample with the largest diameter (rhizolith 5), the microtransects showed scattering $\delta^{13}C_{carb}$ values around –10.9‰ with no trend of increasing or decreasing values from the center to the margin of the tube (Fig. 6-5a).

3.4 Portions of secondary carbonate

Using the stable carbon isotopic signatures of primary loess $CaCO_3$ sampled in the same depth as rhizoliths (–2.4‰), rhizolith carbonate and the $\delta^{13}C_{vegetation}$ (–25.9‰) of root residues in the rhizoliths, portions of secondary carbonate, i.e. carbonate with C originating from root-derived CO_2, were calculated according to Eq. 1.

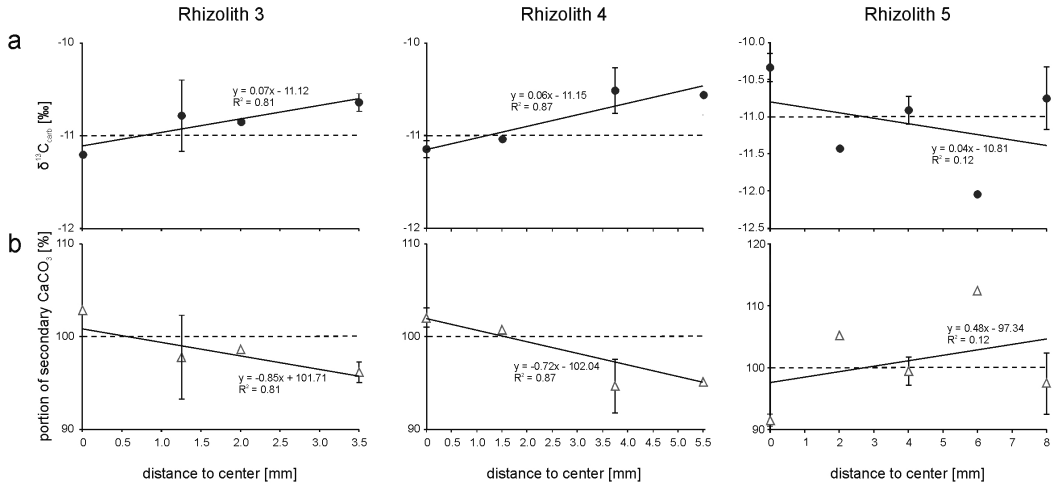

Fig. 6-5. Microtransects of three rhizolith samples. (a) $\delta^{13}C$ values of rhizolith carbonate, measured in microtransects from the center of the carbonatic tube to the margin; dashed line represents the value of rhizolith $\delta^{13}C_{org}$ + 14.9‰, (b) portions of secondary carbonate in rhizoliths. Diameters of rhizolith samples 3, 4 and 5 were 7, 11 and 16 mm, respectively. Error bars (partly smaller than symbol size) show SEM between replicates.

Portions of secondary carbonate were high in rhizoliths (microtransects: 91.4 – 112.5%; Fig. 6-5b) and decreased to values below 12% in loess adjacent to rhizoliths (rhizosphere loess 1; Fig. 6-3c). Reference loess was presumed to contain no secondary carbonate (Fig. 6-

3c). The portion of enclosed primary loess carbonate in rhizolith carbonate averaged 1.9 ± 1.1% in all rhizoliths. Here it is important to mention, that this is the calculated contribution of primary carbonates to the rhizoliths based on their $\delta^{13}C$ values. However, as the sensitivity of this approach based on ^{13}C natural abundance is less than 5%, we conclude that nearly no primary carbonates were present in rhizoliths and the recrystallization of carbonate was close to 100%. Therefore, it is correct to apply radiocarbon dating to estimate the age of the rhizoliths.

3.5 Radiocarbon (^{14}C) ages

The calibrated ^{14}C age of the root OM (rhizolith C_{org}) recovered from the inner part of the rhizoliths was 3150 ± 59 years. The rhizolith carbonate revealed a slightly higher radiocarbon age of 3788 ± 59 years (Tab. 6-1). Hence, carbonate was approximately 640 years older than C_{org} of the same rhizolith.

Table 6-1

Radiocarbon ages of C_{carb} and C_{org} in a rhizolith sampled 1.3 m below the present soil surface from the loess-paleosol sequence at Nussloch.

sample material	^{14}C age, uncal. BP [years]	^{14}C age, cal. BP (2σ range) [years]	^{14}C age, cal. BP, mean ± 1σ [years]
rhizolith C_{carb}	3510 ± 45	3729 - 3847	3788 ± 59
rhizolith C_{org}	2969 ± 34	3091 - 3209	3150 ± 59

4 Discussion

4.1 Stable carbon isotopic composition

The difference between $\delta^{13}C$ values of rhizolith C_{org} and rhizolith C_{carb} averaged at 15.0‰ (Fig. 6-4), thereby strongly suggesting root-derived CO_2 as the main or sole source of the rhizolith C_{carb} (Cerling, 1984). $\delta^{13}C$ values of rhizolith C_{carb} further indicate C_3 plants as source vegetation of the calcified roots (Nordt et al., 1996) and almost no incorporation of carbonate originating from loess (Alonso-Zarza, 1999; Łącka et al., 2009), which is typically characterized by higher $\delta^{13}C$ values.

Rhizolith $\delta^{13}C_{org}$ values (–25.9 ± 0.5‰) were slightly lower than those of loess C_{org} in the Nussloch loess section ranging mainly between –25 and –24‰ (Hatté et al., 1999). Both rhizolith and loess OM derive from C_3 vegetation.

Portions of secondary carbonate decreased in smaller rhizoliths from the center towards their outer boundary (rhizolith 3 and 4; Fig. 6-5). This is in agreement with the fact that a high pCO_2 related to rhizosphere respiration is maintained only at the root surface and decreases within few millimeters distance (Hinsinger et al., 2003). Moreover, primary mineral grains, i.e. also primary loess carbonate with higher $\delta^{13}C_{carb}$ values, occur mainly in the outer parts of the rhizoliths (Fig. 6-2a, d). A trend of decreasing portions of secondary carbonate from the center towards outer parts was, however, not visible in the rhizolith with the largest diameter (rhizolith 5). On the contrary, values in this rhizolith strongly varied and showed a broad spectrum of secondary carbonate portions between 91.4 – 112.5% (Fig. 6-5). As shown by

micromorphology, these fluctuations may be caused by the influence of smaller roots adjacent to the main root which were either not calcified or calcified and "incorporated" into the main rhizolith (see smaller channels in Fig. 6-2a–c). In general, high secondary carbonate portions in rhizoliths (>> 90%) indicate that almost complete isotopic exchange occurred during rhizolith formation by dissolution of primary loess $CaCO_3$ and reprecipitation of carbonate with CO_2 produced by root and rhizomicrobial respiration.

4.2 Radiocarbon ages and rhizolith conservation

The ^{14}C ages of rhizolith C_{org} (3150 years) and C_{carb} (3790 years) revealed Holocene formation of the rhizolith, in contrast to a late Pleistocene age of surrounding loess (17–20 ka; Antoine et al., 2001).

Contrary to carbonate coatings used for determination of the time frame of secondary carbonate formation by radiocarbon dating of innermost and outermost layers (Pustovoytov et al., 2007b), rhizoliths lack distinct layers (Fig. 6-1c, 6-2a–d). Hence, the C_{carb} age of the bulk rhizolith sample is an average age and has to be considered as minimum age for this rhizolith sample from Nussloch. However, in contrast to formation periods of hundreds to thousands of years for secondary carbonate distant from roots (e.g. pseudomycels, loess dolls; Chen and Polach, 1986; Pendall et al., 1994; Kuzyakov et al., 2006), rhizoliths might form within considerably shorter periods, even during the lifetime of the plant and the initial decay of its residues. This is related to the high CO_2 concentration in the rhizosphere and transpirational pull of the plant, leading to preferred Ca^{2+} and HCO_3^- mass flow towards the root (Gocke et al., 2010b).

Radiocarbon ages of rhizolith C_{carb} and C_{org} both were in the same order of magnitude, indicating that rhizolith carbonate was formed in isotopic equilibrium with root- and rhizomicroorganism-derived CO_2 without considerable contamination by introduction of C of other ages. In older secondary carbonates (> tens of thousands of years) even small contamination with modern ^{14}C (< 10%) can yield radiocarbon ages that lead to underestimations in the range of thousands of years (Deutz et al., 2002). In contrast, theoretical incorporation of 10% of modern ^{14}C in the rather young Nussloch rhizolith would reduce its true carbonate age only by 470 years. However, the slightly higher age of the rhizolith carbonate compared to included C_{org} indicates that the rhizolith carbonate was not appreciably contaminated with younger C related to post-segregational alteration. The lack of post-segregational alteration was also confirmed by the slightly increasing $\delta^{13}C_{carb}$ values towards the outer parts of rhizoliths (Fig. 6-5a), which is probably related to the incorporation of primary carbonate grains (Fig. 6-2a, d).

Assuming that the root was encrusted by secondary carbonate during its lifetime within a few years to a few decades at maximum, the difference between ages of the rhizolith carbonate and enclosed OM ($\Delta \approx 640$ years) probably results from the occlusion of a minor portion of primary carbonate from loess. Previously, this has been referred to as "limestone dilution effect" (Williams and Polach, 1971). However, it appears more likely that in secondary carbonate this results from mechanical admixtures of lithogenic carbonate

(Amundson et al., 1989; Monger et al., 1998) or by microbial decomposition of ^{14}C-depleted OM (Wang et al., 1994). Based on calibrated radiocarbon ages of the rhizolith sample, and assuming an age of the surrounding loess carbonate of > 70 000 years (the upper limit for radiocarbon dating), the portion of occluded old carbonate in the radiocarbon dated rhizolith sample was 7.4%.

Mainly, two factors are known that may inhibit carbonate alteration by post-segregational processes (Chen and Polach, 1986; Amundson et al., 1994): dry climatic conditions and high density of the carbonate material. Climatic reasons can be ruled out in the humid region of Nussloch (Heidelberg: 804 mm mean annual precipitation, www.klimadiagramme.de, accessed 02.12.10). Rhizoliths showed high carbonate contents around 85% and at the same time considerable porosities between 15 and 35% (Fig. 6-2a–d), which is higher than porosity of micritic nodules (Khormali et al., 2006; Pietsch and Kühn, 2009). We suggest that high carbonate contents and compactness of the investigated rhizoliths together with good drainage properties of loess might have led to conservation of the rhizolith carbonate. Moreover, after decay of the roots, the CO_2 concentration decreased very fast in the vicinity of the former root, and without high CO_2 concentration and water uptake by roots, no further carbonate movement and recrystallization occurred around the rhizoliths (Hinsinger, 1998). This circumstance also supports the suggestion that rhizoliths are formed during the lifetime of plant roots and not after decay of the root tissue, as assumed by other authors (e.g. Joseph and Thrivikramaji, 2006).

4.3 Implications for rhizolith formation in loess

According to the model of Cerling (1984, 1999), the portion of primary carbonate should be almost zero in all secondary carbonates, because CO_2 fluxes from root respiration are 2–3 orders of magnitude higher than rates of carbonate accumulation. This leads to complete replacement of primary C_{carb} with root-respired C during several cycles of carbonate dissolution and reprecipitation. Cerling (1999) and Monger et al. (1998), however, mentioned that primary carbonate can be occluded in secondary carbonates either in a mechanical or chemical way, thereby leading to wrong interpretation of the carbon isotopic signature of secondary carbonates.

So far, it is unclear whether a complete C_{carb} exchange had happened during rhizolith formation. Due to adjacency to the rhizosphere, this special form of secondary carbonate might be formed in much shorter periods (years to decades; Gocke et al., 2010b) than non-concretional secondary carbonates (hundreds to thousands of years; Kuzyakov et al., 2006; Gocke et al., 2010b). If primary carbonate is present in the parent material (e.g. loess) to a large extent, fast precipitation of secondary carbonate might lead to incomplete dissolution of primary carbonate material. Thus inherited detrital carbonate in secondary carbonate concretions can yield too old radiocarbon ages of the secondary carbonate. Moreover, loess commonly contains primary carbonate not only as $CaCO_3$, but also as dolomite ($CaMg[CO_3]_2$) which can comprise up to ~ 10% of loess weight (Pye and Sherwin, 1999). During dissolution of primary loess carbonate and reprecipitation of secondary rhizolith

carbonate around the roots, lower solubility of dolomite compared to calcite might have led to occlusion of dolomite grains, resulting in a slight overestimation of the age of the rhizolith carbonate. This suggestion is supported by the slightly higher radiocarbon age of rhizolith C_{carb} when compared to rhizolith C_{org}, as well as the occurrence of primary mineral grains (also $CaCO_3$) mainly in the outer part of the rhizoliths (Fig. 6-2a, d).

Rhizoliths of one horizon can have different ages, thus ruling out general conclusions about the age of rhizolith formation (Ziehen, 1980). Pustovoytov and Terhorst (2004) showed for a loess profile near Nussloch that calcified root cells in upper horizons can be older than those obtained from deeper horizons (9 ky in 0.6 m versus 6 ky in 3 m). Isotopic signatures in our study suggest the absence of contamination of rhizolith carbonate by post-segregational incorporation of C of younger age. This means also that sparite, a feature of recrystallisation that occurs in the rhizoliths (Fig. 6-2e), cannot be much younger than the surrounding micritic carbonate. Radiocarbon ages of the calcified roots in the region south of Heidelberg (Pustovoytov and Terhorst, 2004; this study) provide correct chronologic information about the time of their formation. So far, only three ^{14}C ages for calcified roots are available for the region of SW Germany, all of them indicating Holocene age. As the data base is very low and the three ^{14}C ages coincide with different stratigraphical units, general conclusions regarding the climatic conditions promoting rhizolith formation in loess are not possible. Nevertheless, we speculate here that root calcification occurs mainly during periods of rather dry climatic conditions and/or moisture regimes with strong seasonality. For a rhizolith sample from South African dune sands, Cramer and Hawkins (2009) reported a carbonate ^{14}C age of 7.7 ka. The authors concluded that the rhizolith was formed either during a warm, dry period (the "Holocene altithermal") or shortly before the onset of this period. Further investigations are required to allow for statements about climatic drivers forcing the formation of rhizoliths.

4.4 Implications of the rhizolith – loess macrotransects

C_{carb} as well as C_{org} contents were considerably larger in rhizoliths than in loess, which was shown by both the secondary nature of rhizolith carbonate and the comparatively good preservation of rhizolith OM due to encrustation by carbonate.

Living roots can generate remarkable amounts of exudates, and decaying root biomass can lead to a notable input of OM in soil or sediment (Kuzyakov and Domanski, 2000; Nguyen, 2003). Due to a large abundance of rhizoliths in the Nussloch loess section (app. 10 – 20 rhizoliths m^{-2}) it can be stated that rhizodeposits contributed a considerable portion of post-sedimentary OM in the loess. Differentiation between rhizosphere loess and reference loess was, however, not possible based on C_{org} and C_{carb} contents (Fig. 6-3a).

Portions of secondary carbonate, calculated from $\delta^{13}C_{carb}$, indicated that pedogenic processes were also effective in loess distant to visible root remains. However, these values are not suitable to quantify the input of root-derived OM in rhizosphere loess. Based on lipid analyses, Gocke et al. (2010a) showed that microbial degradation of OM was stronger in rhizosphere loess when compared to rhizoliths and reference loess which was attributed to stronger microbial activity in this former rhizosphere due to exudates and root fragments. As

degradation leads to ^{13}C enrichment in C_3 roots (Wiesenberg et al., 2004), highest $\delta^{13}C_{org}$ values should occur in rhizosphere loess when compared to rhizoliths and loess. This trend was visible only in one of the macrotransects (rhizolith 1; Fig. 6-3b), however without significant differences. Very likely, changes of $\delta^{13}C_{org}$ values in rhizosphere loess, caused by addition of unknown portions of stronger microbially degraded OM to the original synsedimentary OM, are too small to be detected by isotope ratio mass spectrometry. Thus, these $\delta^{13}C_{org}$ values, too, can neither be used to differentiate between loess adjacent to roots and root-free loess, nor to determine the amount of root biomass that was incorporated post-sedimentary. Furthermore, the variability of $\delta^{13}C_{org}$ values of rhizoloess in horizontal transects (> 1‰) leads to the question, how meaningful low differences in loess $\delta^{13}C_{org}$ values within a vertical transect (< 1‰) of the sedimentary sequence are (Hatté et al., 1999). Lipid analyses of rhizolith and loess OM provide a tool for the elucidation of these uncertainties: Based on two different lipid molecular proxies, Gocke et al. (2010a) demonstrated the incorporation of considerable amounts of root-derived OM in loess adjacent to rhizoliths: 111% and 70% in rhizosphere loess 1 (0–2.5 cm) and 11% and 5% in rhizosphere loess 2 (2.5–5 cm).

4.5 Chronologic implications for paleoenvironmental reconstructions

In the region south of Heidelberg, maximum ages of 9 ka for rhizoliths and calcified root cells show that they formed in the Holocene (Pustovoytov and Terhorst, 2004; this study), while surrounding loess was deposited mainly during the last glacial–interglacial cycle (Zöller et al., 1988; Hatté et al., 1998). At Nussloch, rhizoliths from a depth of 1.3 m are considerably younger with 3 ka when compared to surrounding loess which was deposited between 17 ka and 20 ka before present (Antoine et al., 2001).

For terrestrial paleoclimate archives other than loess it has been recognized that properties of the original, synsedimentary OM may have been altered by introduction of younger roots. For a Chinese peat sequence, Zhou et al. (2005) calculated a contamination of up to 16% of modern C caused by post-sedimentary incorporation of younger plant biomass. Similar conditions can occur in loess-paleosol sequences (Head et al., 1989). Liu et al. (2007) suggest an older source for the contamination of the synsedimentary plant signal in loess: OM of the source material of loess. Our current results (this study; Gocke et al., 2010a), however, suggest that an admixture of younger, i.e. post-sedimentary OM is more likely. This can entail serious problems concerning the reliability of ^{14}C ages of loess OM (Hatté et al., 1998; Rousseau et al., 2007). Consequences for bulk $\delta^{13}C_{org}$ might be negligible, if both the synsedimentary and the younger organic materials derived from C_3 vegetation, and if the only purpose of $\delta^{13}C_{org}$ measurement is the determination of the photosynthetic pathway of vegetation. However, reconstruction of paleoprecipitation from loess $\delta^{13}C_{org}$ (Hatté and Guiot, 2005), based on small variations of $\delta^{13}C$ values, might be disturbed by post-sedimentary input of root-derived C_{org}, which is not necessarily uniform throughout complete loess-paleosol sequences.

Considerable age discrepancies (> 10 ka) are possible also between secondary carbonate and surrounding loess (Pustovoytov and Terhost, 2004; this study). This problem might be

important also for other loess-paleosol sequences. It was not perceived by Wang et al. (2000), who studied secondary carbonates in loess-paleosol sequences on the Chinese Loess Plateau and in central North America. The authors reported a considerably wider spectrum for the difference between stable carbon isotopic composition of secondary carbonate and of OM from surrounding loess than the difference of ~ 15‰ suggested by Nordt et al. (1996). These differences in $\delta^{13}C$ values were linked to monsoonal and paleo-El Niño variations, whereas the true reason may be the different source vegetation of secondary carbonate and loess OM.

It is still a prevalent belief that total OM and secondary carbonate formed synsedimentary in loess (e.g. Becze-Deák et al., 1997; Wang et al., 2000, 2004). So far, only few studies mentioned the possibility that secondary carbonate formation in loess-paleosol sequences might have occurred later than sedimentation, however without testing this idea by comparison of radiocarbon ages of rhizoliths and synsedimentary loess C_{org} (Boguckyj et al., 2009). The presumption was confirmed by first radiocarbon data from calcified roots from loess-paleosol sequences (Pustovoytov and Terhorst, 2004; Cramer and Hawkins, 2009; this study). Whereas in the present study, C_{org} contents were similar in loess distant and adjacent to rhizoliths, it was also possible to elucidate differences in the molecular composition of the OM therein as determined for lipid content and lipid composition between rhizoloess and reference loess (Gocke et al., 2010a). It was observed that remarkable amounts of root-derived OM were incorporated in loess at least up to a distance of 5 cm from rhizoliths and possibly even in larger distances. Together with the fact that rhizoliths were formed later and by a different vegetation than loess OM, this might entail uncertainties for bulk analyses in loess OM, as e.g. $\delta^{13}C_{org}$ and radiocarbon dating.

5 Conclusions

Based on elemental and isotopic C analyses on organic and carbonatic compounds of loess and rhizoliths (calcified roots) from the loess-paleosol sequence at Nussloch, SW Germany, we conclude that

- rhizoliths were formed by C_3 vegetation;
- during formation of the rhizoliths, C from primary loess carbonate was almost completely exchanged with C from CO_2 respired by roots and rhizosphere microorganisms, leading to portions of secondary carbonate of nearly 100% as derived from $\delta^{13}C$ data;
- rhizoliths were generated fast: during the lifetime of the roots as obtained from radiocarbon data;
- carbonate material of the rhizoliths was not recrystallized post-segregationally, and is therefore suitable for radiocarbon dating;
- rhizoliths are considerably younger than surrounding loess ($\Delta \approx 14$ ka at minimum) (^{14}C, luminescence dating); and
- post-sedimentary root penetration into loess probably led to an overprint of the original signal of loess OM, which, however, cannot be estimated by means of C_{org} contents or stable carbon isotopic composition.

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Study 7: Rhizoliths in loess – evidence for post-sedimentary incorporation of root-derived organic matter in terrestrial sediments as assessed from molecular proxies

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Abstract

Loess-palaeosol sequences are important terrestrial archives for studying Quaternary climate change. A major assumption for palaeoenvironmental reconstruction based on loess organic matter (OM) is that it represents the signal of syn-sedimentary incorporated plant biomass, i.e. predominantly grass vegetation. However, recent studies on rhizoliths (roots encrusted by secondary carbonate) formed in loess reveal the possibility of post-sedimentary penetration of loess-palaeosol sequences by deeply rooting plants. This likely led to incorporation of younger root-derived OM into surrounding loess, potentially causing an overprinting of the initial plant-derived signal of loess OM.

To obtain information on the source vegetation of rhizoliths and surrounding loess OM we examined rhizoliths and loess from Nussloch, SW Germany, using alkane and fatty acid (FA) molecular proxies. Moreover, the lipid composition was compared in transects from rhizoliths via rhizosphere loess towards root-free loess for a preliminary assessment towards quantifying the post-sedimentary input of rootderived OM in loess.

Loess OM gave a combined signal from shoot and root biomass from grass vegetation, confirming the assumption of deposition during glacial periods with scarce grass vegetation cover. In contrast, the lipid composition of rhizolith OM reflected roots of woody vegetation, indicating the post-sedimentary character of rhizoliths. Stronger degradation of OM, together with a large content of microorganism-derived FAs in the former rhizosphere indicated rhizodeposition associated with high microbial activity in loess adjacent to rhizoliths, at least up to a distance of 5 cm. Rhizosphere loess and reference loess at a distance of 50–70 cm showed a significantly different OM composition, thereby revealing the incorporation of considerable portions of root-derived OM into loess in the vicinity of roots. Further studies are necessary for an exact quantification of this potential overprint of the syn-sedimentary loess OM, which might cause uncertainty in palaeoenvironmental studies.

1 Introduction

Soils of arid and semiarid regions show favourable conditions for precipitation of secondary carbonate (Birkeland, 1999), which takes place in isotopic equilibrium with soil CO_2 (Cerling, 1984) released by way of root respiration and organic matter (OM) decomposition. Rhizoliths are a particular form of secondary $CaCO_3$ formed by encrustation of plant roots. While the organic part of the root is mainly degraded during decomposition, tubular carbonatic structures remain in the sediment. They occur locally in sandy and silty calcareous sediments (Ziehen, 1980; Becze-Deák et al., 1997), usually separately from other types of secondary carbonate accumulations (Becze-Deák et al., 1997).

Rhizoliths were first mentioned as ‘Osteokollen‘ [see review by Ziehen (1980)] because of their bone-like structure (Greek osteon = bone, Latin collon = stem). Due to the variability in their morphology and occurrence in a variety of different settings, numerous names have been used for rhizoliths [e.g. rhizocretion, root cast; summarized by Klappa (1980)] and

distinct hypotheses for their genesis have been discussed. Calcified root structures can be a precursor of rhizogenic calcretes, i.e. calcretes formed mainly by root activity (Wright, 1989).

Rhizoliths have been frequently described. However, it is a somewhat novel approach to use them as a proxy for palaeoenvironmental reconstruction, although they are probably very sensitive environmental recorders (Becze-Deák et al., 1997). Such reconstruction based on calcified roots has focussed on the carbonate (carb) material, either by way of macro- and micromorphological studies (Becze-Deák et al., 1997; Alonso-Zarza and Arenas, 2004), or stable carbon and oxygen isotopic analysis ($\delta^{13}C_{carb}$, $\delta^{18}O_{carb}$; Kuleshov and Gavrilov, 2001; Alonso-Zarza and Arenas, 2004; Pustovoytov and Terhorst, 2004; Wang and Greenberg, 2007).

The vegetation under which rhizoliths were formed has been scarcely examined. In some early studies, they were attributed exceptionally to coniferous trees, whereas others reported calcified roots additionally for other woody vegetation like peach and birch (Ziehen, 1980). Jaillard and Callot (1987) described recent calcified roots mainly under grassland and herbaceous plants, whereas they were rarely observed under pine and possibly under wine plants (B. Jaillard, personal communication). However, according to Ziehen (1980), determination of the source vegetation is impossible if the calcified roots are no longer connected to the plant. A preliminary source apportionment was tried on the basis of the shape and size of rhizoliths and root features in calcretes. For instance, Wright et al. (1988) concluded from the morphology of palaeosol calcretes from the UK and Spain that 'relatively small plants' prevailed during formation of the secondary carbonate concretions. Except for determination of the photosynthetic pathway of source vegetation of rhizoliths using $\delta^{13}C_{carb}$ (see above), to the best of our knowledge no further analytical methods have been performed, which focus on a more detailed characterization of the former vegetation. We hypothesize that part of the OM derived from root tissue must be preserved within and beneath rhizoliths in loess, because secondary carbonate encrustation leads to protection of organic remains from the former root tissue against degradation by microorganisms. The molecular composition of OM within rhizoliths should provide evidence of the former vegetation; to the best of our knowledge this has not been tested. Biomarker analysis using diagnostic molecular proxies could help determine the plant origin of OM because of their chemotaxonomic significance (Maffei, 1996a,b; Rommerskirchen et al., 2006; Wiesenberg and Schwark, 2006). Numerous organic compounds typical of individual plant species, e.g. suites of terpenes in pine trees, occur in plants. After plant death, degradation of biomass results in typical degradation products in soils and sediments, which can be used as biomarkers (Peters et al., 2005). For grass, such specific biomarkers have not been observed. Nevertheless, molecular proxies obtained from the distribution of fatty acids (FAs), alcohols and alkanes have been found to be of chemotaxonomic significance for differentiating grasses from woody plants (Maffei, 1996a,b), or among grasses following different photosynthetic pathways (C_3 vs. C_4; Rommerskirchen et al., 2006; Wiesenberg and Schwark, 2006). Alkanes in loess have in particular been used for source apportionment of the vegetation (Xie et al., 2003; Liu and Huang, 2008; Bai et al., 2009), but the approach has been rarely attempted with FAs and alcohols (Xie et al., 2003). Because of the presumed lower recalcitrance of FAs and alcohols

(Bol et al., 1996; Wiesenberg et al., 2004b, 2008) in loess, when compared to alkanes, the number of studies is limited. Nevertheless, the cold and arid climatic conditions prevailing during loess accumulation enable good preservation of molecular proxies (Kuder and Kruge, 1998; Xie et al., 2004). Additionally, encrustation of root tissue by $CaCO_3$ provides improved preservation of OM in rhizoliths. In this study, we investigated for the first time lipids and molecular proxies deriving from FAs and alkanes in rhizolith OM and loess in Central Europe.

As rhizoliths occur in terrestrial sediments, it can be assumed that root-derived OM was incorporated during the lifetime of the roots in adjacent soil and sediment, the so called rhizosphere. This can be expected as the rhizosphere of living roots is a hot-spot of fine roots, root growth, and nutrient and water acquisition (Jones, 1998). Hence, close to large rhizoliths, residues of fine roots and root exudates, as well as associated microorganisms that lived in the former rhizosphere, can be expected. This has been ignored in past studies of loess-palaeosol sequences. While it was clearly shown that root exudates consist mainly of low molecular weight organic components (Bertin et al., 2003), it can be assumed that the recalcitrance of these organic compounds in loess–palaeosol sequences is limited as a result of their high solubility and susceptibility to being consumed by microorganisms. Hence, organic remains of microorganisms feeding on exudates rather than the exudates themselves might have the potential to be preserved in the former rhizosphere. While many authors suggest that rhizolith formation occurred syn-sedimentary with loess deposition (Becze-Deák et al., 1997; Wang and Greenberg, 2007), recent studies reveal that roots, as well as calcareous root features, in loess-palaeosol sequences can be significantly younger than surrounding loess (Pustovoytov and Terhorst, 2004). Hence, rooting of younger plants must have significantly changed the loess OM in the vicinity of the roots, not only by incorporation of root-derived OM itself, but additionally by cometabolic degradation of initial loess OM by microorganisms feeding on root exudates and root remains. However, the amount of post-sedimentary root-derived OM incorporated into terrestrial sediments is unknown. Particularly for Quaternary continental sediments with high chronological resolution, which are used for palaeoenvironmental reconstruction, this uncertainty can entail problems for the interpretation of the stable carbon isotopic composition of OM ($\delta^{13}C_{org}$) as a proxy for palaeovegetation, palaeoprecipitation and other palaeoecological conditions (e.g. Hatté and Guiot, 2005). Some authors have already mentioned the problem of post-sedimentarily incorporated biomass in terrestrial sediments (Head et al., 1989; Zhou et al., 2005). Attempts to correct the bulk organic signals are scarce (Liu et al., 2007) or resulted in fractionation of the bulk carbon pool and the assumption that only particulate OM (90–300 lm) reflects the original C_{org} signal (Head et al., 1989; Zhou et al., 2005). Most authors assume that OM enters buried terrestrial sediments mainly via dissolved organic carbon, whereas incorporation of root-derived OM has not been discussed. We hypothesize that a considerable amount of root-derived OM is incorporated into terrestrial sediments (e.g. loess) adjacent to roots and that this can be quantified by comparing the molecular composition of root and rhizosphere materials (remaining root biomass and organic material in the sediment) with that of sediments of the same horizon distant from roots.

In the region of Heidelberg, SW Germany, rhizoliths with a diameter of up to 5 cm occur locally abundant in dune sands at Sandhausen (Löscher and Haag, 1989), as well as in the loess-palaeosol sequence at Nussloch, with a high abundance in the Nussloch loess section (ca. 10–20 rhizoliths m^{-2}). These rhizoliths were formed by C_3 vegetation, as shown by the stable carbon isotopic composition of organic carbon ($\delta^{13}C_{org}$ –25.9 ± 0.5‰) and inorganic carbon ($\delta^{13}C_{carb}$ –10.9 ± 0.1‰; Gocke et al., unpublished results). So far, only one rhizolith radiocarbon age (3150 yr b.p.; Gocke et al., unpublished results) is available from Nussloch, indicating post-sedimentary formation of this rhizolith. However, rhizoliths from the same depth might have formed during different time intervals (Ziehen, 1980), thereby impeding general conclusions about the chronological context between terrestrial sediments and calcified roots. The aim of this study was to elucidate the origin of these rhizoliths using molecular markers. Comparison of the composition of lipid fractions in rhizoliths and loess parent material should provide information concerning the sources of both materials, thereby providing evidence whether rhizolith formation took place simultaneously with loess deposition or was a post-sedimentary occurrence. Moreover, we aimed to quantify the input of root-derived material to the rhizosphere. We therefore analysed several rhizoliths and horizontal transects from these rhizoliths to loess distant from roots for their molecular composition with respect to FAs and alkanes.

2 Materials and methods

2.1 Sampling

Rhizolith and loess samples were collected from a late Pleistocene loess-palaeosol sequence at the open cast mine of HeidelbergCement AG, Nussloch, SW Germany (49.19°N, 8.43°E, 217 m above sea level) from a depth interval between 2.2 and 2.6 m below the present surface. The recent soil had a depth of 0.8 m, so samples were taken 1.4–1.8 m below the recent soil. The loess-palaeosol sequence, with a total thickness of 18.5 m, did not show any sign of recent pedogenesis in the sampled interval. Loess from the profile had a total organic carbon (C_{org}) content of < 5 mg g^{-1} and high C_{carb} content of 34 mg g^{-1} (Bente and Löscher, 1987), leading to a high pH ($CaCl_2$) of 8.1. For a more detailed description of the sampling site see Antoine et al. (2001). For two rhizoliths (R), loess transects were sampled from the former root (i.e. rhizolith) towards root-free loess at distances from 0–2.5 cm and 2.5–5 cm from R and were named rhizoloess (RL1 and RL2, respectively). Reference loess (L), which was free of any visible root remains, from the same depth interval and the same stratigraphic unit, was sampled at a distance of 50–70 cm from R. Both rhizoliths had a length of 20–40 cm, a diameter of 2–4 cm and were of similar morphology.

All replicate results in the following represent separate analyses of two different rhizoliths and corresponding transects towards reference loess.

2.2 Elemental and lipid analyses

Rhizoliths were rinsed with deionized water to remove adhering loess, and all samples were dried at 60 °C for 24 h and crushed in a ball mill. C_{org} and C_{carb} contents of rhizoliths and

loess were measured by way of combustion in an oven (Feststoffmodul 1300, AnalytikJena) at 550 °C and 1000 °C, respectively, followed by CO_2 detection with a N/C analyser (AnalytikJena).

Loess (root-free loess, ca. 100 g; rhizoloess, 30–50 g) and rhizolith samples (30–50 g) were extracted for free lipids with dichloromethane (DCM)/MeOH (93:7, v:v; Wiesenberg et al., 2004a) using Soxhlet extraction for at least 40 h. Neutral and FA fractions were obtained by way of chromatographic separation of the extract using solid phase extraction (SPE) with KOH-coated silica gel (Wiesenberg et al., 2004a, 2010). Neutral lipids were eluted with DCM, followed by FAs, which were eluted with DCM/HCO_2H (99:1, v:v). After reducing the solvent volume to dryness, the neutral fraction was dissolved in hexane and separated with respect to aliphatic and aromatic hydrocarbons, as well as low polarity hetero compounds. Separation was performed on columns filled with activated silica gel (100 Å) by eluting aliphatic hydrocarbons with hexane, aromatic hydrocarbons with hexane/DCM (1:1, v:v) and low polarity hetero compounds with DCM/MeOH (93:7, v:v). The FA and aliphatic hydrocarbon fractions in particular afforded interesting results from gas chromatography with flame ionisation detection (GC–FID; Agilent 7890) after addition of deuteriated standards for quantification ($D_{39}C_{20}$ acid, or $D_{50}C_{24}$ alkane, respectively). FAs were derivatized using BSTFA [*N,O*-bis(trimethylsilyl)trifluoroacetamide] prior to GC, while aliphatic hydrocarbons were directly amenable to GC–FID analysis. Identification of compounds and determination of possible co-elutions were performed via GC–mass spectrometry (GC–MS) analysis (HP 5890 chromatograph coupled to a HP 5971 spectrometer) and correlation with external standard mixtures.

2.3 Molecular proxies

2.3.1 Carbon preference index

Long chain FAs in fresh biomass from higher plants typically reveal a strong predominance of even homologues (Tissot and Welte, 1984), expressed in the carbon preference index (CPI_{FA}):

$$CPI_{FA} = [(\sum n\text{-}C_{20\text{-}32\ even} / \sum n\text{-}C_{19\text{-}31\ odd}) + (\sum n\text{-}C_{20\text{-}32\ even} / \sum n\text{-}C_{21\text{-}33\ odd})] / 2 \quad (1)$$

While fresh plant biomass usually has CPI_{FA} values > 4, degradation after sedimentation and microbial reworking result in values close to 1 (Cranwell et al., 1987), because of preferential decomposition of even homologues and a contribution from odd homologues, e.g. of wax esters or other potential precursor compounds with long chain alkyl FAs, during degradation.

The CPI for alkanes (CPI_{Alk}) has a similar meaning to CPI_{FA}, except it relates to a predominance of odd homologues and contains primary wax remains of plants as well as degradation products of different compound classes including, e.g. alcohols, FAs and wax esters:

$$CPI_{Alk} = [(\sum n\text{-}C_{25\text{-}35\ odd} / \sum n\text{-}C_{24\text{-}34\ even}) + (\sum n\text{-}C_{25\text{-}35\ odd} / \sum n\text{-}C_{26\text{-}36\ even})] / 2 \quad (2)$$

Odd *n*-alkanes are dominant components of plant leaf waxes (Eglinton et al., 1962; Kolattukudy et al., 1976), whereas even homologues derive mainly from degradation of OM. Degradation of plant OM results in a decrease in the odd predominance (Zhou et al., 2005). CPI_{Alk} values < 10 indicate degradation of OM (Cranwell, 1981) or can be related to root biomass (Wiesenberg et al., unpublished results). In addition, microbial OM contains mainly short chain alkanes and lacks an even or odd predominance. Therefore, CPI_{Alk} values around 1 are characteristic of highly degraded OM and/or abundant microbially-derived OM (Cranwell, 1981; Zhou et al., 2005).

2.3.2 Average chain length (ACL)

The ACL of FAs (ACL_{FA}) indicates whether OM in sediments is derived predominantly from microbial (values < 20) or plant biomass (values > 20), because long chain FAs (> C_{20}) are produced exclusively by plants, whereas short chain FAs can be derived from plants and microbial biomass (Kolattukudy et al., 1976). ACL_{FA} is calculated as follows:

$$ACL_{FA} = \sum(z_n \text{ x } n) / \sum(z_n) \quad (3)$$

where n is the number of carbons and z_n the amount of the FA with n carbons, with n in the range 12–32.

Analogous to ACL_{FA}, the ACL of alkanes (ACL_{Alk}) indicates the degradation of plant and microbial biomass as well as microbially-derived OM (Bray and Evans, 1961). The contribution of long chain plant-derived components is indicated by high values ≥ 23 (Eglinton et al., 1962; Kolattukudy et al., 1976), while the presence of microbially-derived OM is expressed by lower values. ACL_{Alk} is calculated as

$$ACL_{Alk} = \sum(z_n \text{ x } n) / \sum(z_n) \quad (4)$$

where n is the number of carbons and z_n the amount of alkanes with n carbons, with n in the range 12–15.

2.4 Statistical analyses

Mean values and standard errors of the mean are presented for replicate analyses. Differences between rhizolith, rhizoloess and loess were tested to be significant using a t-test for dependent samples with a significance level of $\alpha = 0.1$. Statistical analysis was carried out using STATISTICA for Windows (version 7.0, StatSoft Inc., Tulsa, USA).

3 Results and discussion

3.1 Bulk carbon and lipid content

The C_{org} content decreased strongly from 72 ± 9 mg g^{-1} in rhizoliths (R) to 10 ± 1 mg g^{-1} in rhizoloess (RL) 1 and 6 ± 1 mg g^{-1} in RL2. The C_{carb} content was 92 ± 2 mg g^{-1} for R, but only 46 ± 1 mg g^{-1} for RL 1 and 44 ± 1 mg g^{-1} for RL2 (corresponding to $CaCO_3$ contents of 76 ± 1%, 38 ± 0.4% and 37 ± 0%). C_{org} and C_{carb} contents of reference loess (L) were slightly lower (4 ± 2 mg g^{-1} and 40 ± 3 mg g^{-1}, respectively; Fig. 7-1A) than the RL values, without significant differences between RL and L.

The extractable lipid content normalized to sample weight was 168 ± 13 µg g^{-1} for R and immediately dropped towards 62 ± 5 µg g^{-1} for RL. For all RL samples the extractable lipid content was almost identical. For L the lipid content was lowest (48 ± 7 µg g^{-1}), but did not significantly differ from RL (Fig. 7-1B). The lipid content of all samples was lower than that of recent surface soils (ca. 300–600 µg g^{-1}; Amblès et al., 1994; Lichtfouse et al., 1995; Wiesenberg et al., 2006), but almost comparable to B horizons of recent soils (20–90 µg g^{-1}; Wiesenberg et al., 2006). The lower lipid content of loess can be attributed to the generally low content of OM in loess. At Nussloch, C_{org} content of rhizoloess, as well as reference loess sampled at a distance of 50–70 cm from the rhizolith, was between 4 and 10 mg g^{-1}, which is in a similar range to literature data from Nussloch (5 mg g^{-1}; Bente and Löscher, 1987). The content of extractable lipids of R was 2.5 times as high as for RL and three times as high as for L (Fig. 7-1B). This is attributed mainly to larger initial input of OM in rhizoliths by way of root growth when compared to loess, which contains OM from a presumably sparse grass vegetation cover present during deposition (Bai et al., 2009). Additionally, encrustation of the root tissue by secondary carbonate might have led to improved preservation of root material.

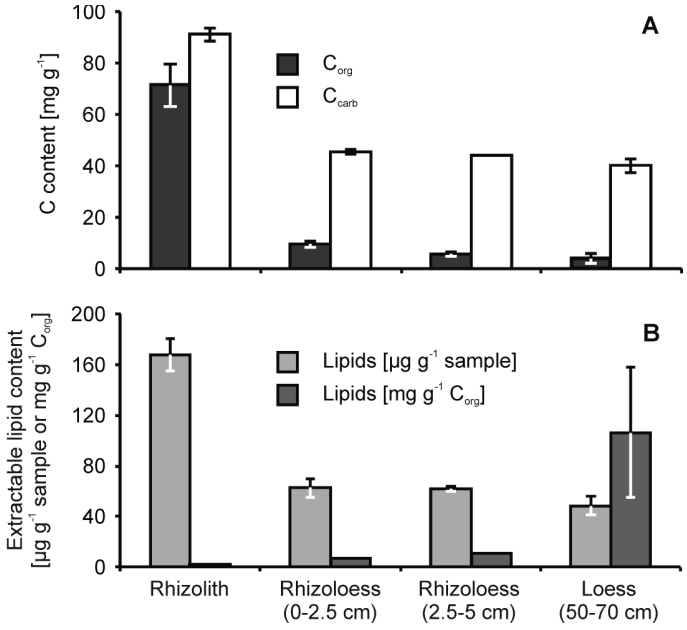

Fig. 7-1. Content of (A) C_{org} and C_{carb} and (B) extractable lipids in rhizolith, rhizoloess and loess. Lipid content was normalized to bulk sample and C_{org}. Distance of loess samples from rhizoliths is given in parentheses.

Normalized to C_{org} content, the amount of extractable lipids was lowest in R (2.4 ± 0.1 mg g^{-1} C_{org}), increased in RL (6.9 ± 1.4 mg g^{-1} C_{org} in RL1 and 10.9 ± 1.2 mg g^{-1} C_{org} in RL2) and was highest in L (106.5 ± 51.7 mg g^{-1} C_{org}; Fig. 7-1B). Extractable lipids comprised 1–2% of total biomass for fresh root tissue (ca. 2.2–4.4% of C_{org}; Wiesenberg, 2004 and unpublished data), whereas the portion was considerably higher for above-ground plant parts (4–10% of biomass or ca. 8.9–22.2% of C_{org}; Wiesenberg, 2004; Wiesenberg et al., 2008 and unpublished data). This suggests that lipids in rhizoliths and rhizoloess might be of root origin, with a very low contribution from extractable lipids and preservation of more complex OM structures including, e.g. lignin (Marschner et al., 2008). In contrast, the highest lipid

contents related to C_{org} in loess indicate above- and belowground biomass as the source for loess OM. The large contribution of extractable lipids to C_{org} in loess (106.5 ± 51.7 mg g^{-1}) is even larger than for recent soils (Wiesenberg et al., 2006) and is related to the strong degradation of precursor compounds since sedimentation, which is connected with a selective enrichment in degradation products like alkanes (see below).

3.2 Molecular composition

Besides distribution patterns of FAs and alkanes, several molecular proxies were found to be useful in elucidating the source of the OM in loess, rhizoloess and rhizolith samples. They include the sum of mono-unsaturated FAs (MUFAs; $C_{14:1}$, $C_{16:1}$, $C_{18:1}$), the sum of poly-unsaturated FAs (PUFAs; $C_{16:2}$, $C_{18:2+3}$), the preference of even/odd long chain FAs (CPI_{FA}), the preference of odd/even long chain alkanes (CPI_{Alk}) and ACL_{FA} and ACL_{Alk}.

3.2.1 FAs

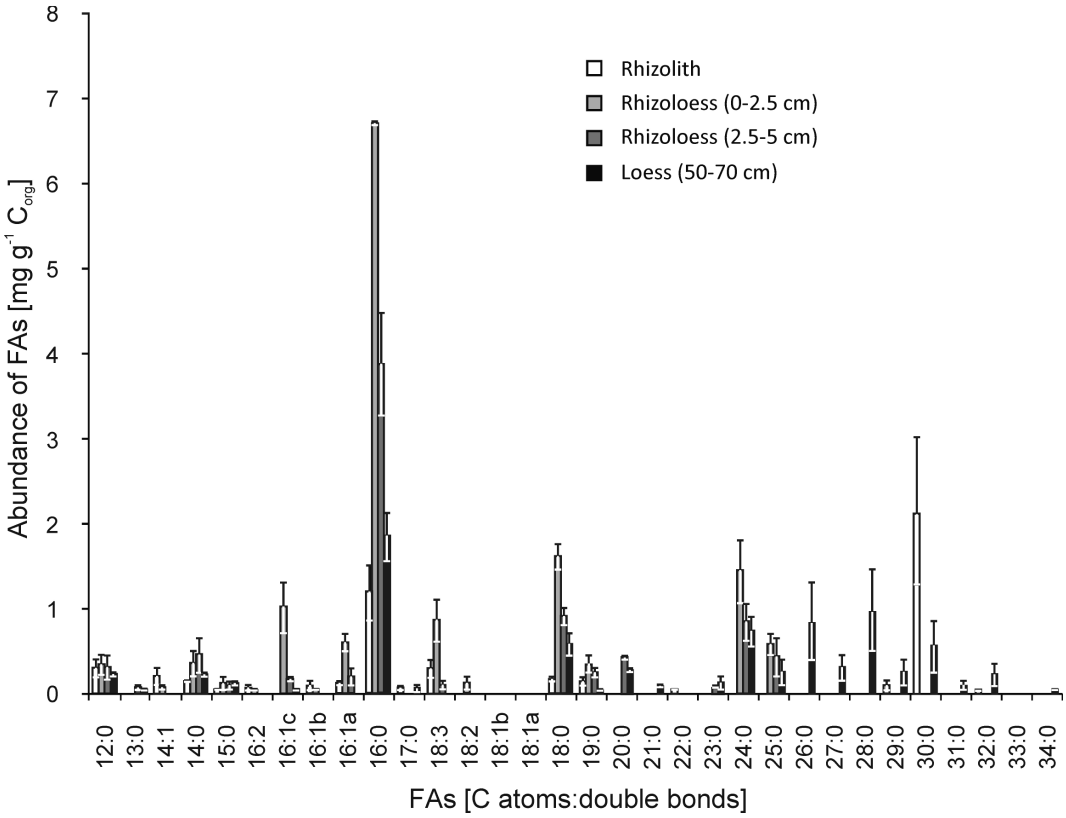

Fig. 7-2. FA distribution in rhizolith, rhizoloess and reference loess; FA amount is normalized to C_{org} (mg g^{-1} C_{org}).

Distribution patterns of FAs in both loess and rhizolith samples showed a dominance of $C_{16:0}$ and long chain homologues (> $C_{17:0}$), as well as a predominance of even/odd long chain FAs. Rhizosphere loess distribution patterns were similar to those of R and L, but lacked long chain homologues > $C_{25:0}$ (Fig. 7-2). In general, the FA distributions confirmed the composition of plant-derived OM in terrestrial environments (Xie et al., 2003). This was confirmed by high CPI_{FA} values in L (4.0 ± 0.5) and R (7.6 ± 1.2). Significantly lower ($p < 0.05$) CPI_{FA} values were obtained for RL (1.7 ± 0 in RL1 and 1.4 ± 0 in RL2), indicating

a stronger degradation and/or microbial remains dominating the source of the FAs in the rhizosphere.

$C_{14:1}$ and $C_{16:1+2}$ unsaturated FAs are mainly attributed to microbial OM (Kolattukudy et al., 1976; Harwood and Russell, 1984). The contribution of these components to total FAs increased from rhizolith (8.3 ± 1.6%) to loess adjacent to rhizoliths (0–2.5 cm; 11.5 ± 1.8%) and decreased at a larger distance towards the reference loess (rhizoloess 2.5–5 cm: 4.4 ± 0.5%; loess: 1.6 ± 0.4%; Fig. 7-3). Poly-unsaturated $C_{18:2+3}$ and long chain FAs (≥ 20:0) are attributed to higher plant-derived OM (Kolattukudy et al., 1976; Harwood and Russell, 1984) and contributed most to the total FAs in rhizoliths (48.0 ± 2.0%). These contributions were significantly lower ($p < 0.005$) in rhizoloess (0–2.5 cm: 19.4 ± 3.5%, 2.5–5 cm: 21.7 ± 1.6%, respectively) than in rhizoliths. In loess the contribution of these plant-derived FAs was in a similar range as in rhizoliths (46.2 ± 11.7%). In particular, the abundance of PUFAs successively decreased with increasing distance from rhizolith (5.8 ± 0.1%) towards reference loess (0%). The remaining short chain FAs ($C_{12:0–19:0}$, $C_{18:1}$), attributed to both microbial and higher plant biomass (Kolattukudy et al., 1976), increased from rhizoliths (43.6 ± 3.8%) to rhizoloess (0–2.5 cm: 69.0 ± 5.3; 2.5–5.0 cm: 73.9 ± 2.1) and dropped towards reference loess (52.1 ± 11.3%).

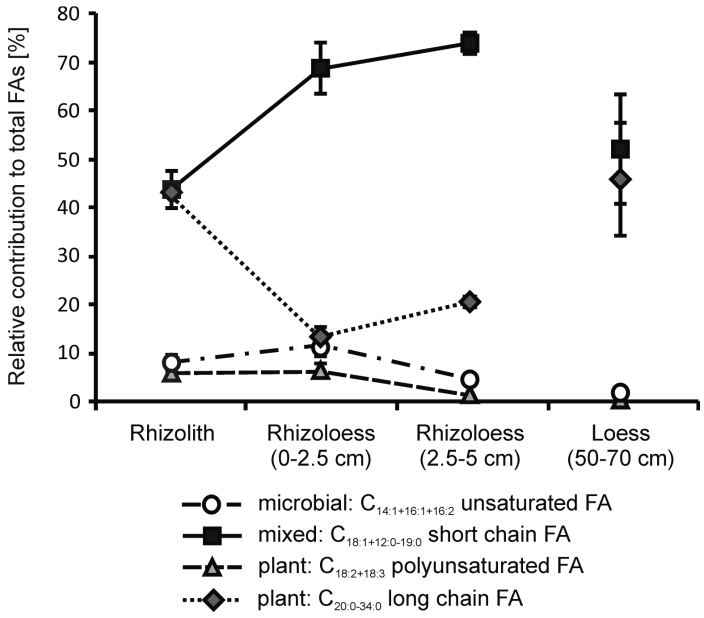

Fig. 7-3. Relative contribution of FAs of different origin (microbial and higher plant as well as mixed sources) to total FA fraction for rhizoliths and corresponding transects via rhizoloess towards reference loess.

The FA distribution of rhizolith OM, with a high abundance of $C_{16:0}$ and low abundance of very long chain homologues (> $C_{26:0}$), with the exception of very high amounts of $C_{30:0}$, might indicate its root origin, as suggested by the morphology (Klappa, 1980; Ziehen, 1980). Even long chain FAs (> $C_{19:0}$) were most abundant in loess, except for $C_{30:0}$, which showed much higher content in R than L. The most abundant long chain FA was $C_{28:0}$ in L, but was $C_{24:0}$ in rhizoloess and $C_{30:0}$ in rhizolith samples. Therefore, the contribution of plant-derived biomass and/or its corresponding degradation remains must be different in these sample types. Most

likely, the large increase in $C_{24:0}$ FA from L towards RL derives from rhizodeposits in the vicinity of roots and/or more intensive degradation of plant biomass than in loess and rhizolith. This is confirmed by the high abundance of microorganism-derived FAs in the rhizoloess as, indicated by MUFA, CPI_{FA}, and additionally exceptional low values of ACL_{FA}. Like CPI_{FA} (Fig. 7-4A), ACL_{FA} values were highest in R (21.8 ± 0.4) and slightly lower in L (20.3 ± 1.6), with RL samples having the lowest values (17.8 ± 0.3 in RL1 and 17.6 ± 0.1 in RL2; Fig. 7-4B). While fresh plant biomass is commonly characterized by high ACL_{FA} and CPI_{FA} values, degradation results in decreasing values (Peters et al., 2005). Additionally, the contribution of microbial biomass itself and of microbial reworking is expressed in very low ACL_{FA} and CPI_{FA} values (Cranwell, 1981). Hence, the highest CPI_{FA} and ACL_{FA} values for R are related to plant-derived OM, which is less degraded than the OM in L with intermediate values. The very low CPI_{FA} and ACL_{FA} values in RL are attributed to the relatively high abundance of microbially-derived OM near R, i.e. in the former rhizosphere, as previously described only for recent soils and living plants (Jones, 1998). As the former rhizosphere was presumably a hot-spot for microbial activity during the root lifetime (Coleman, 1994) the microbial remains near former roots reflect these conditions even in ancient systems.

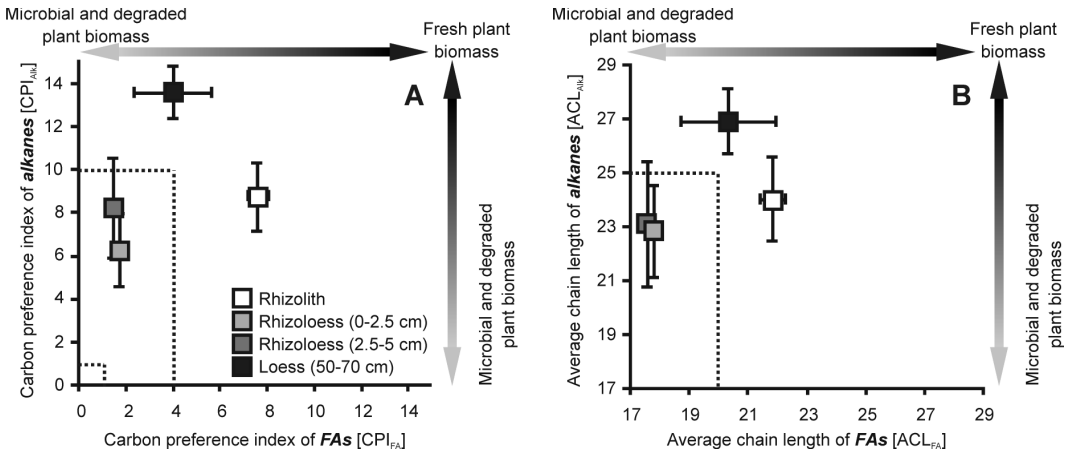

Fig. 7-4. Comparison of (A) CPI_{Alk} and CPI_{FA} and (B) ACL_{Alk} and ACL_{FA} in rhizoliths, rhizoloess and loess. (A) Dashed lines indicate areas of pure microbial OM (CPI_{Alk} and $CPI_{FA} \approx 1$) and area of strongly degraded OM ($CPI_{Alk} < 10$, $CPI_{FA} < 4$; Cranwell, 1981; Cranwell et al., 1987; Xie et al., 2003; Zhou et al., 2005). (B) Dashed line indicates area of OM with large microbial contribution ($ACL_{Alk} < 25$, $ACL_{FA} < 20$; Kolattukudy et al., 1976).

However, the distribution pattern itself does not allow us to quantitatively estimate the contribution of root-derived OM in rhizoloess samples, because the FA composition did not reveal a consistent trend from R via RL towards L (Fig. 7-3). This is related to different contributions from plant and microbial origins in these transitions: In the former rhizosphere, the abundance of microorganisms is supposed to be high during the plant's lifetime as they commonly feed on fragments of dead root biomass and the exudates of roots during the lifetime of plants (Coleman, 1994; Jones, 1998). In contrast, the former root tissue (rhizoliths) and sediment (loess) are clearly dominated by plant-derived OM (Fig. 7-3).

For estimating rhizomicrobial- and root-derived lipids in rhizoloess, quantities of MUFAs and PUFAs were compared for different sample types.

The PUFAs, which are readily degradable because of the number of double bonds (Kawamura et al., 1980), are commonly not regarded in terrestrial sediments like loess, where, e.g. microbial activity and oxygen exposure, might lead to their rapid disappearance. Analogous to lacustrine sediments (Kawamura et al., 1980), the presence or absence, as well as the quantity, of PUFAs in loess are indicators of the incorporation of plant-derived OM on one hand and the level of its degradation and preservation on the other hand. PUFA content normalized to extract yield in L was below detection limit (50 ng g^{-1} C_{org}). The amounts were slightly higher in RL2 (0.009 ± 0.005 mg g^{-1} C_{org}), significantly increased towards RL1 (0.133 ± 0.022 mg g^{-1} C_{org}) and were highest in R (0.191 ± 0.068 mg g^{-1} C_{org}; Fig. 7-5). This clear differentiation, with two end members (loess and rhizolith) and rhizoloess values in between, was used for the quantification of root-derived input, assuming that R represents the upper limit in the sample set with 100% root-derived OM. L did not contain PUFAs and was therefore used as the other end member with 0% root-derived PUFAs. The root-derived PUFAs in rhizoloess were calculated as follows:

$$\text{portion of root-derived PUFA [\%]} = [\Sigma\ PUFA_{Rhizoloess} / (\Sigma\ PUFA_{Rhizolith} - \Sigma\ PUFA_{Loess})] \times 100 \quad (5)$$

The portion of root-derived PUFA was lowest in distant rhizoloess (4.9%) and significantly increased in rhizoloess near rhizoliths (69.7%).

As MUFAs are mainly microorganism-derived (especially some of the $C_{16:1}$ and $C_{18:1}$) but also partly plant-derived (Harwood and Russell, 1984), we use the term ‘‘rhizomicrobial-derived MUFA” for both root and associated microbial biomass sources. MUFA content normalized to extract yield was lowest in L and RL2 distant from roots (2.5–5 cm), with 0.010 ± 0.005 mg g^{-1} and 0.034 ± 0.007 mg g^{-1}, and higher in RL1 close to the rhizoliths and in R (0.243 ± 0.013 mg g^{-1} and 0.221 ± 0.090 mg g^{-1}; Fig. 7-5).

Analogous to PUFAs (Eq. 1) the rhizomicrobial-derived MUFAs in rhizoloess were calculated:

$$\text{portion of rhizomicrobial-derived MUFA [\%]} = [\Sigma\ MUFA_{Rhizoloess} / (\Sigma\ MUFA_{Rhizolith} - \Sigma\ MUFA_{Loess})] \times 100 \quad (6)$$

According to MUFA, the amount of rhizomicrobial-derived OM accounts for 110.9% in RL1 adjacent to rhizoliths and 11.5% in the distant RL2.

Very low amounts of MUFAs and missing PUFAs in loess clearly show the comparatively severe degradation of loess OM. Consequently, increasing amounts of MUFA and PUFA from L over RL towards R clearly showed the notable contribution of less degraded, potentially younger OM to loess OM in rhizosphere, especially close to roots (Fig. 7-5). In general, the lower abundances of root-derived PUFAs vs. rhizomicrobial-derived MUFAs can be explained first by a predominantly plant-derived origin of PUFAs and second by an admixture of microorganism-derived remains in MUFAs as well as a stronger degradation of PUFAs in rhizoloess because of the larger number of double bonds. However, both the contribution of root- and microorganism-derived OM in RL1 close to R (70% for PUFA and 111% for MUFA) and the significantly lower contribution in RL2 distant from the rhizoliths (5% and 11%, respectively) may represent minimum values since unsaturated FAs are

comparatively susceptible to degradation (Kawamura et al., 1980). The high abundance of MUFAs is most likely related to microorganisms that lived in the former rhizosphere during the lifetime of the roots – similar to the situation in soil (Coleman, 1994), leading to an accumulation of rhizodeposit remains, as well as remains of the microbial biomass which fed on the rhizodeposits. This might explain larger MUFA content in RL1 than in R (Fig. 7-5).

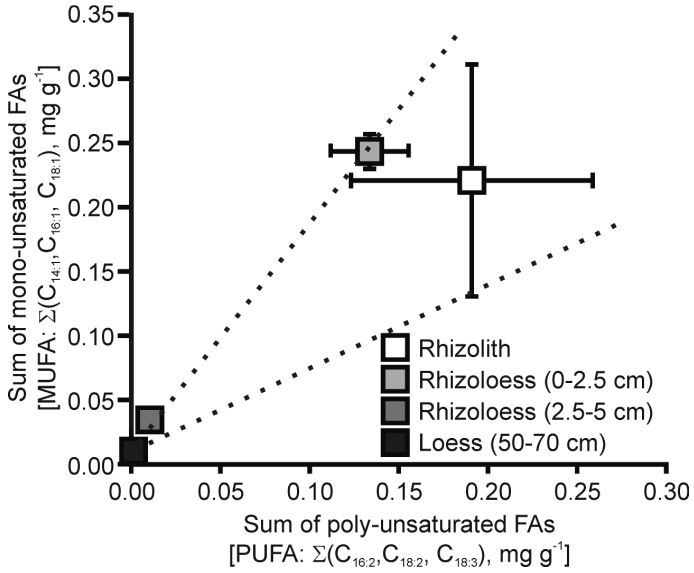

Fig. 7-5. Abundances of MUFAs and PUFAs in rhizoliths, rhizoloess and loess, normalized to total lipid content (mg g^{-1} extract). Dashed lines represent area of mixing between loess OM and rhizolith OM. Values outside this zone indicate a significant accumulation of additional microbial biomass and degradation products, especially in RL1.

Assessing the exact contribution of root-derived OM in RL requires examination of other molecular proxies that are less influenced by degradation like, e.g. lignin (e.g. Marschner et al., 2008). In addition, knowledge of the incorporation mechanisms of FAs in the rhizosphere is at present limited (Wiesenberg et al., 2010). Nevertheless, the FA molecular proxies clearly indicate that the post-sedimentary input of root- and rhizomicrobial-derived OM to loess and other terrestrial sediments is an important factor influencing the content and composition of the OM in the sediment.

3.2.2 Alkanes

C_{org}-normalized amount of alkanes was greatest in L and lower in RL and R. The whole sample set was characterized by a typical terrestrial higher plant distribution, with a predominance of long chain odd homologues (C_{25+}; Fig. 7-6; Eglinton et al., 1962; Kolattukudy et al., 1976). This was confirmed by CPI_{Alk} which was highest in L (13.6 ± 1.0) and lowest in RL1 (6.3 ± 1.9). Values for RL2 and R were intermediate and were similar to each other (8.2 ± 1.1 and 8.8 ± 3.6, respectively), with slightly higher values for R (Fig. 7-4A).

The most abundant *n*-alkanes in R were either C_{29} or C_{31}. In RL the most abundant homologue was the same as in the corresponding R, whereas in loess it was always *n*-C_{31}. In earlier studies, alkane patterns maximizing at *n*-C_{31}, as well as *n*-C_{33}, were attributed to grass

vegetation, whereas *n*-C_{29} was not related to a specific vegetation type but could be produced by either grasses or woody vegetation (Maffei, 1996a,b). Within the short chain homologues ($C_{15–24}$), *n*-C_{16}, *n*-C_{18} or *n*-C_{20} were slightly enriched compared to other homologues, which was especially apparent in rhizoloess. Abundances of all alkanes in RL were intermediate between the corresponding homologues in R and L (Fig. 7-6).

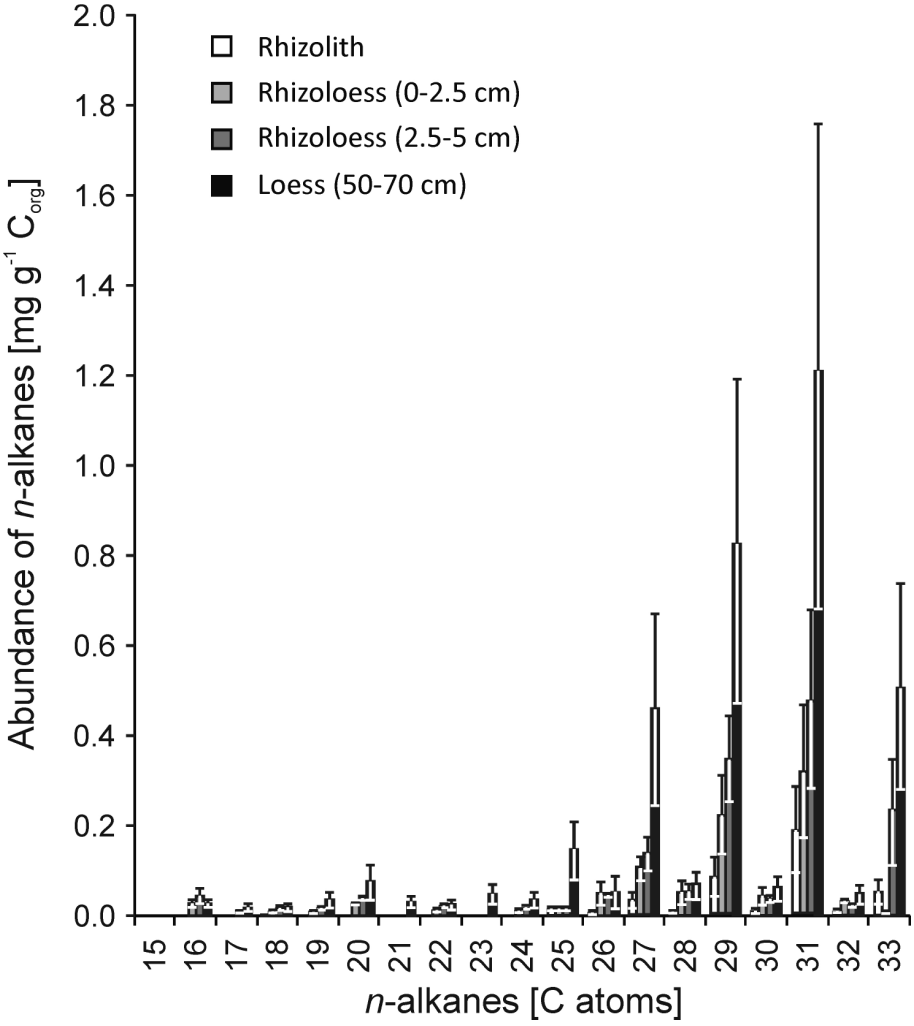

Fig. 7-6. *n*-Alkane distribution in rhizolith, rhizoloess and reference loess; amounts of alkanes are normalized to C_{org} (mg g^{-1} C_{org}).

In R, 77.8 ± 7.2% of the alkanes were contributed by plant-derived, odd long chain homologues ($C_{25–33}$; Eglinton et al., 1962), while the proportion was lower in RL1 (63.5 ± 4.4%; Fig. 7-7). At a larger distance from R the relative contribution of these alkanes increased from 68.1 ± 9.2% in RL2 towards 87.5 ± 1.4% in L. The relative abundance of odd long chain alkanes was significantly ($p < 0.05$) different in L, R and RL. The relative amount of even long chain alkanes ($C_{26–32}$) varied only slightly in the sample set (between 6.6 ± 0.1 in L and 14.3 ± 2.9 RL1). The highest contribution was observed in rhizoloess and decreased in the order RL1 > RL2 > R > L. Similarly, like the even long chain alkanes, the contribution of short chain alkanes ($C_{15–24}$) was highest in RL samples (22.7 ± 9.6% in RL1 and 22.2 ± 7.2%) and decreased both towards R (14.2 ± 6.9%) and L (5.9 ± 1.5%).

Based on alkane and FA distributions, the OM in the L samples most likely relates to C_3 grass vegetation, whereas rhizoliths were probably formed by woody vegetation like C_3 shrubs or trees. However, determination of the plant species was not possible because of limited knowledge of the lipid composition of root tissue, especially of trees and shrubs.

ACL_{Alk} values in loess (26.9 ± 1.2; Fig. 7-4B) were comparable to those of higher plant biomass (25–31; Eglinton and Hamilton, 1967). Values were lowest in RL (22.9–23.1), with almost identical values close by (RL1) and distant from rhizoliths (RL2). R was characterized by intermediate values between L and RL (24.1 ± 1.6).

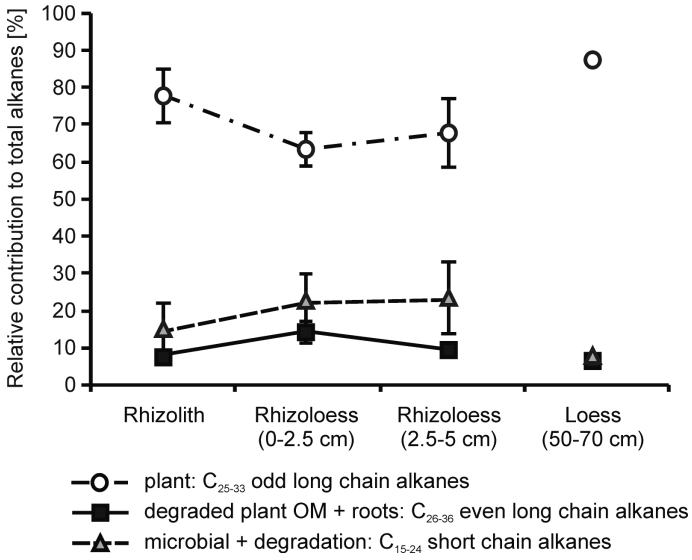

Fig. 7-7. Relative contribution of alkanes from different origins (microbial and higher plant, as well as degradation products) to total *n*-alkane fraction for rhizoliths and corresponding transects via rhizoloess towards reference loess.

The highest values of ACL_{Alk} and CPI_{Alk} were always obtained for loess, indicating the higher plant origin of the OM (Bray and Evans, 1961; Castillo et al., 1967; Poynter et al., 1989). As demonstrated by FA patterns (Fig. 7-2), R was also formed by higher plants. However, slightly lower ACL_{Alk} and CPI_{Alk} in R than in L, together with different long chain alkane maximum (Fig. 7-6) suggest unequal biogenic sources for OM in R and L. Low ACL_{Alk} values in the rhizosphere are most likely attributed to the contribution of microbial remains (Kolattukudy et al., 1976; Harwood and Russell, 1984). In rhizoliths and loess, microbial remains are also present, as shown by $C_{16:0}$ and $C_{18:0}$ FAs (Fig. 7-2), but this source of OM is masked by the prevalent plant biomass. Low CPI_{Alk} values in RL confirm this and show that even the RL2 distant from R was obviously rooted or received root exudate. This loess was consequently part of the former rhizosphere, where root remains were incorporated and microbial degradation of roots and sedimentary OM took place. This is strongly connected to the hot-spot theory, which hypothesizes greatest microbial activity and hence strongest degradation of OM in soil adjacent to the tips of fine roots (Jones, 1998).

3.3 Implications for rhizolith formation in loess and possible consequences for palaeoenvironmental reconstruction

Molecular proxies showed that rhizoliths in Nussloch derived from vegetation other than loess OM. The latter afforded a combined signal from syn-sedimentary shoot and root biomass with high amounts of long chain FAs ($C_{22–32}$). This reflects former steppe conditions with grass vegetation (e.g. Wiesenberg and Schwark, 2006), in agreement with loess sedimentation taking place during glacial periods. In contrast, rhizolith OM contained lower amounts of very long chain FAs ($C_{26–30}$), more likely reflecting roots from shrub or tree vegetation. In general, it is believed that the vegetation cover during loess deposition was minor and consisted mainly of grass (Bai et al., 2009). This suggests that rhizoliths were not formed syn-sedimentary with loess deposition, but roots entered the loess later, followed by calcification during their lifetime (Gocke et al., unpublished results). In many palaeoenvironmental studies based on rhizoliths and other types of pedogenic carbonate in loess-palaeosol sequences, the chronological context of secondary carbonate nodules is not mentioned. Commonly, authors link them to the age of surrounding sediment or soil (e.g. Wang and Follmer, 1998; Wang et al., 2000; Becze-Deák et al., 1997). However, the hypothesis of post-sedimentary rooting of loess was enforced by the Holocene age (3150 yr b.p.) of one rhizolith from Nussloch (Gocke et al., unpublished results). In contrast, surrounding loess was deposited during the Pleistocene, between 20 and 17 ka (Antoine et al., 2001). The possible existence of such age discrepancies between sediment and calcified roots has only been recognized by a few authors (Pustovoytov and Terhorst, 2004; Cramer and Hawkins, 2009).

Moreover, the above rhizolith sample from Nussloch revealed nearly identical radiocarbon ages for C_{org} and C_{carb} (Gocke et al., unpublished results). This indicates that root calcification took place during plant lifetime and was not a fossilisation process. Lambers et al. (2009) related $CaCO_3$ precipitation adjacent to roots to mass flow induced by the transpirational pull of the living plant. CPI_{FA} and ACL_{FA} values in particular provide further evidence for this theory by showing the relatively low degradation of rhizolith OM (higher values) when compared to loess OM (lower values; Fig. 7-4A, and B), probably as a result of improved protection of the former root tissue by carbonate encrustation in advance of substantial decay of root biomass.

While deep rooting of loess by grass plants is unlikely because of their root morphology, shrubs and trees are able to penetrate sediments up to 5 m and more (Canadell et al., 1996). It is known for recent soils that living roots can generate huge amounts of exudate and that dead root biomass can lead to a remarkable input of OM (Kuzyakov and Domanski, 2002; Nguyen, 2003). It is therefore likely that, during warmer phases connected with pedogenesis, root-derived OM was also incorporated into parts of the loess deeper than the corresponding soil, and that sediment below this soil could be affected by deeply rooting plants.

Lipid distribution patterns (Figs. 7-2 and 7-6) indicate that modification of OM by post-sedimentary processes, including abiotic degradation and microbial decomposition, is strongest in loess and slightly lower in rhizoloess and rhizolith. On the other hand, the

abundance of microbial remains was higher in RL than L, as indicated by CPI_{Alk}, ACL_{Alk} and the presence of unsaturated FAs in RL. This supports the idea of stronger degradation of plant remains by microbial reworking in the former rhizosphere, leaving relatively larger portions of microbial remains therein. Based on PUFAs, the minimum contribution of root-derived OM to loess OM of 69.7% was calculated for RL at a distance of 0–2.5 cm to R. A notable post-sedimentary contribution of root-derived OM at greater distances from rhizoliths is likely and should be estimated in future studies. Particularly for terrestrial sediments, which are often poor in C_{org}, post-sedimentary input of OM might lead to a remarkable overprint of the composition of the original (i.e. synsedimentary) OM. In rhizoloess no visible remains of roots and no obvious colour change vs. the surrounding sedimentary material were observed, suggesting that the post-sedimentary contribution of root-derived OM might have been underestimated in previous studies. This could explain some uncertainties in bulk C_{org} radiocarbon (^{14}C) ages and $\delta^{13}C_{org}$ measurements in loess-palaeosol sequences (Hatté et al., 1999; Rousseau et al., 2007). It was assumed previously that $\delta^{13}C_{org}$ values might represent mixed signals from syn-sedimentary prevailing vegetation and additional sources. This additional OM was thought to be either inherited from the source material of loess (Liu et al., 2007) or incorporated from percolating soil solutions of modern soil (Zhou et al., 1997). Post-sedimentary penetrating roots themselves, however, were never discussed as a possible origin. This preliminary study is the first to demonstrate considerable post-sedimentary contribution of root-derived OM to terrestrial sediments. For the first time, an attempt was made to quantify this overprint using organic geochemical analysis. Further investigations, and from other sites, are necessary to elucidate the impact of post-sedimentary incorporated OM in loess-palaeosol sequences as well as the environmental conditions which lead to formation of rhizoliths and related rhizodeposits.

4 Conclusions

For the loess-palaeosol sequence at Nussloch, SW Germany, we have shown that the remains of OM in rhizoliths are suitable for elucidating their formation and influence on the surrounding sediment. Comparison of FA and alkane distributions in loess and rhizoliths suggested grass biomass as the source for loess OM, in agreement with loess deposition during glacials, with scarce vegetation cover. Root biomass of shrubs or trees was found to be the origin of the rhizoliths. This, together with radiocarbon data from the same section, disproves the prevalent opinion about rhizolith formation taking place simultaneously with loess sedimentation. While loess sedimentation occurs under steppe-like conditions, roots entered the loess later, probably under different climatic conditions, and consequently derive from different vegetation.

Lipid analysis combining several FA and alkane proxies, including CPI and ACL, revealed information regarding the contribution of post-sedimentary incorporated plant biomass and the intensity of degradation of plant-derived biomass. Based on PUFAs, we quantified the amounts of root-derived OM incorporated into loess adjacent to rhizoliths by comparison with reference loess from the same depth, which accounted for at least 70% of the

OM adjacent to rhizoliths. Furthermore, MUFAs argued for an enrichment of root-derived and associated microbial biomass remains in the vicinity of rhizoliths. ACL and CPI molecular proxies showed that the influence of the former roots by generation of rhizodeposits led to a modification of the OM composition in the rhizosphere, which was notable at least to a distance of 5 cm from the rhizolith. The consequential overprint of syn-sedimentary incorporated loess OM by younger biomass from other sources might entail uncertainties regarding chronological (^{14}C) and palaeoenvironmental ($\delta^{13}C$) studies. Therefore, more research is required to elucidate the influence of larger deeply rooting plants on the composition of OM in the underlying sediment.

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Vergangenheit ist Geschichte,
Zukunft ist Geheimnis,
aber jeder Augenblick ist ein Geschenk.
Tibetisches Sprichwort

Declaration / Erklärung

Herewith I declare that the present work was exclusively prepared by me using no others than the named sources and devices. In addition I declare that I have not yet tried to hand in this PhD thesis, be it successfully or not. I have not definitely failed to pass an equivalent PhD test at another university.

Hiermit erkläre ich, dass ich diese Arbeit selbständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet habe. Ferner erkläre ich, dass ich anderweitig mit oder ohne Erfolg nicht versucht habe, diese Dissertation einzureichen. Ich habe nicht diese oder eine gleichartige Doktorprüfung an einer anderen Hochschule endgültig nicht bestanden.

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