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

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Abstract

The C 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) y, while it was ≈ 600 (365–1600) y 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

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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 y) 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 sec-

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ondary 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 3×10^{-5} d^{-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., 2011). 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., 2010). 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., 2010) 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. 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 its 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 d for wheat and 59 d 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. (2011). 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. 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 d; *Kuzyakov* and *Cheng*, 2004) as well as for isotopic exchange between primary $CaCO_3$ and respired CO_2 (≤ 4 d; *Gocke* et al., 2010), 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 (nonrhizosphere 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.

To measure the amounts of C tracer incorporated into loess $CaCO_3$ by recrystallization, 2 g (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

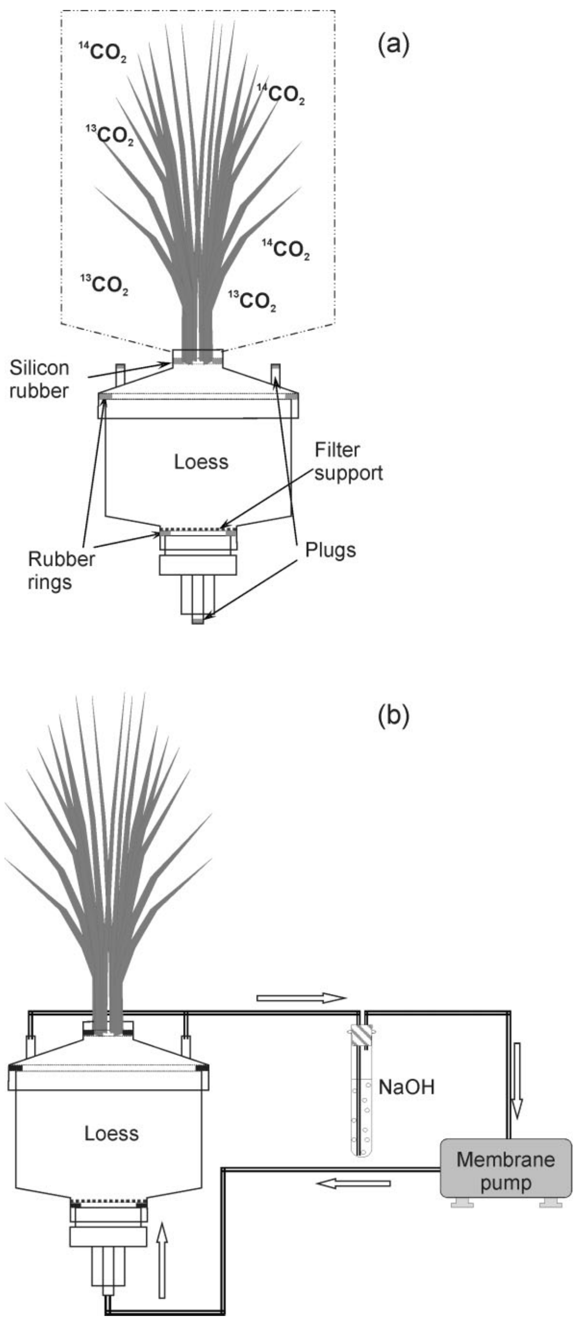

Figure 1: Experimental setup. a) Labeling of aboveground biomass in an airtight chamber with ^{13}C- and ^{14}C-labeled CO_2. b) Trapping of CO_2 released by root and rhizomicrobial respiration in NaOH (modified after *Kuzyakov* and *Siniakina*, 2001).

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 $SrCO_3$ (7×10^{-10}). The $SrCO_3$ precipitant was then purified by centrifugation and washing with deionized 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 70%, measurement error ≤ 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 (*A*; Eq. 1), where *R* is the ^{13}C : ^{12}C ratio of the international PDB reference (*R* = 0.011 237 2). Based on ^{13}C mass balance, the initial recrystallization rate was calculated as 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. 2).

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

$$CaCO_3 \text{ recrystallization rate: } ^{13}C = \frac{A_l^{CaCO_3} - A_{NA}^{CaCO_3}}{\left(A_l^{CO_2} - A_{NA}^{CO_2}\right) \cdot t} \quad (2)$$

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

$$^{14}C_{SA}^{CO_2} = \frac{^{14}C^{CO_2}}{C_t^{CO_2}} \quad (3)$$

$$C_{t_{recryst}}^{CaCO_3} = \frac{^{14}C^{CaCO_3}}{^{14}C_{SA}^{CO_2}} \quad (4)$$

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

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

3 Results

3.1 Recrystallization rates

^{14}C analyses showed that > 99% of the applied $^{14}CO_2$ label was assimilated by the plants during the labeling procedure (*Gocke* et al., 2011). 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. 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 d (Fig. 2a). This corresponds to a mean recrystallization rate of 6.35 × 10^{-5} d^{-1} (Tab. 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. 2a).

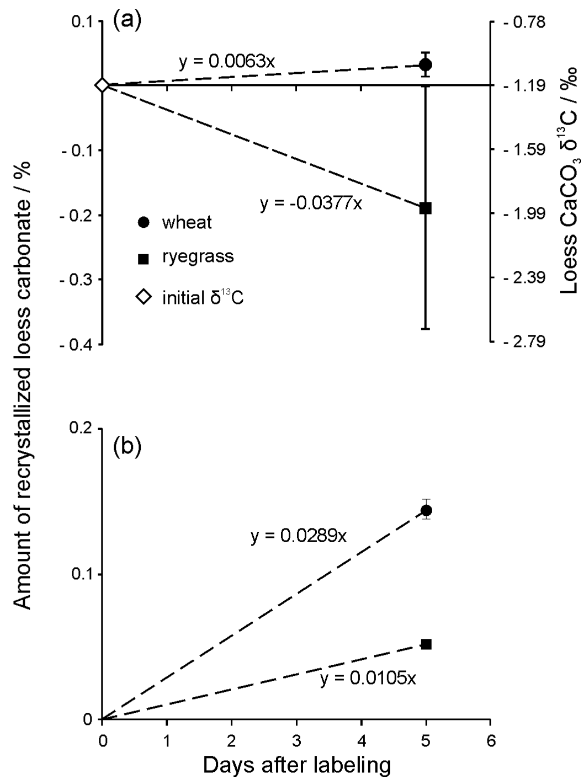

Figure 2: Amounts of recrystallized carbonate (± SE) as a percentage of total loess carbonate 5 d after the labeling, based either on the a) ^{13}C- or b) ^{14}C-labeling approach. For the former, $\delta^{13}C$ values of loess $CaCO_3$ are presented on the right Y-axis. The diagrams show the amounts 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 d. After 5 d, 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. 2b). These amounts correspond to mean rates of 2.89 × 10^{-4} d^{-1} and 1.05 × 10^{-4} d^{-1} under wheat and ryegrass, respectively (Tab. 1).

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.,

Table 1: $CaCO_3$ recrystallization rates in rhizosphere and nonrhizosphere (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. For comparison, ranges of recrystallization rates without plants under CO_2 concentrations between 380 and 50 000 ppm in loess air (*Gocke* et al., 2010b) are also displayed.

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

2006). Therefore, the slopes of the trend curves (Fig. 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:

$$CaCO_3(t) = 100 \cdot \left(1 - \exp\left[-t \cdot rate \cdot \frac{GS}{365}\right]\right), \qquad (6)$$

with *t*: time in years, *rate*: recrystallization rate in d^{-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 y for wheat. Extrapolation of the values from ^{14}C labeling yielded shorter recrystallization periods of 130 and 240 y for wheat and ryegrass, respectively (Fig. 3, Tab. 2).

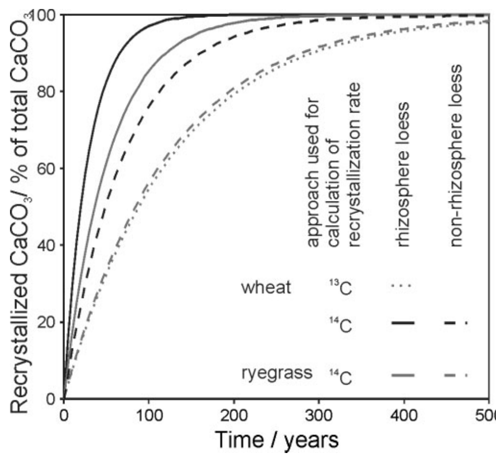

Figure 3: $CaCO_3$ recrystallization periods modeled for rhizosphere loess (continuous lines) based on recrystallization rates estimated by isotopic exchange with ^{13}C (only wheat) or ^{14}C. For comparison, recrystallization periods for nonrhizosphere loess (dashed lines) based on the ^{14}C approach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

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) 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.

Table 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 / y^{-1}	
^{13}C	590 [365–1600]	n.d.*
^{14}C	130 [125–140]	240 [225–255]

* n.d. not determined

4.2 Estimated $CaCO_3$ recrystallization rates

In contrast to previous studies (*Kuzyakov* et al., 2006; *Gocke* et al., 2011), 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 to months) of plant growth leads to linear increase of recrystallized $CaCO_3$. Thus, slopes of Fig. 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: 2.89×10^{-4} d^{-1}, ryegrass: 1.05×10^{-4} d^{-1}), while the ^{13}C approach produced usable results only for wheat (6.35×10^{-5} d^{-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 *vs.* ^{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, Tab. 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. 1). We therefore did not analyze $\delta^{13}C$ in nonrhizosphere 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., 2011): 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 nonrhizosphere 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 6 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. 20 000–120 000 y 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. 1).

Recalculation of the hypothetical increase of $CaCO_3$-$\delta^{13}C$ values based on ^{14}C data (Tab. 1 and Fig. 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 process 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} d^{-1} for nonrhizosphere loess, 10^{-4} d^{-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} d^{-1}) were higher than when using ^{14}C (10^{-6} d^{-1}) (*Gocke* et al., 2010), while the situation was *vice versa* in the current study with rhizosphere loess (10^{-5} d^{-1} for ^{13}C and 10^{-4} d^{-1} for ^{14}C) (Tab. 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 C remaining in water after washing the loess (dissolved inorganic and organic C, [DIC and DOC, respectively]) 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 uncertainties, 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 y were necessary for 99% recrystallization of primary loess carbonate, while the ^{14}C approach yielded a maximum value of 240 y (Fig. 3), with a narrow range between 225 and 255 y (Tab. 2). Taking into account the upper and lower limit of the ^{13}C-based rate (Tab. 1), however, the 99% recrystallization period based on ^{13}C data varies between 365 and 1600 y (Tab. 2). The ^{14}C data also showed that the rates in loess not adjacent to roots are approximately half that in rhizosphere loess (Tab. 1), yielding recrystallization periods of 315 and 555 y for wheat and ryegrass, respectively, in nonrhizosphere loess (Fig. 3).

Due to the uncertainties caused from the experimental design with one isotopic pulse and sampling 5 d 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 C 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 10^3 y (*Becker-Heidmann* et al., 1996). Under semiarid 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 1000–3800 y (*Pendall* et al., 1994). *Pustovoytov* and *Leisten* (2002) demonstrated that after a 1000-y-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. 6000–9000 y BP (*Pustovoytov* and *Terhorst*, 2004), whereas the loess accumulation in this area ceased in the Late Pleistocene (ca. 16 000 y 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 nonrhizosphere loess, leading to considerably shorter recrystallization periods (*Gocke* et al., 2011). These conditions are, however, restricted to few millimeters 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 micrometers) 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} d^{-1} (^{13}C approach) and 10^{-4} d^{-1} (^{14}C approach). Extrapolating the rate estimated by ^{13}C labeling to longer periods indicates that about 600 (365–1600) y 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) y. 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 semiarid environments suggest that a complete cycle of carbonate recrystallization requires $n \times 10^3$ y. Taking into account the slower recrystallization in nonrhizosphere, this supports our estimations under controlled conditions.

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