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 $\approx$ 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$_2^\text{d}$ 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$^6$ 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-
ondary carbonates: δ13C and Δ14C (Pendall et al., 1994) and in dated artificial carbonate material (Pustovoytov and Leisten, 2002). Analysis of 13C 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 CO2 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 14C pulse labeling of plants grown on loess. Based on the isotopic exchange between primary loess CaCO3-C and C from respired CO2, the 14C assimilated by plants, respired by roots and rhizomicrobial organisms, and incorporated in secondary CaCO3 was quantified in the loess CaCO3. 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, 14C and/or 13C 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 14C 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 14C 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 13C and 14C labeling without plants, but by direct contact between primary carbonate (from loess) and dual-labeled (13C, 14C) CO2 in closed system (Gocke et al., 2010). The results argued for the preference of 14C over 13C for studies, because the data calculated based on 14C were more consistent. Recrystallization rates obtained by 14C 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 CO2 supply in planted loess by root and rhizomicrobial respiration. Therefore, we expected that higher recrystallization rates of CaCO3 in the presence of plants will allow also application of 13C 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—13C and 14C—to assess the initial rates of initial carbonate recrystallization by pulse labeling. For this purpose, we labeled plants in atmosphere with 13CO2 and 14CO2 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 (CaCO3 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 CaCO3 content of loess leads to carbonate recrystallization without formation of additional CaCO3, because primary loess calcite represents the major Ca2+ source for secondary CaCO3, while further Ca2+-bearing minerals like feldspar or some mafic minerals (e.g., mica, amphiboles) cannot be weathered in the presence of CaCO3.

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 CO2 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 CO2 released by root and rhizomicrobial respiration. The aboveground plant parts were pulse-labeled simultaneously in 14CO2 and 13CO2 atmosphere, with the 13C-isotopic label consisting of 10 mg of 99% enriched Na213CO3 per plant pot with wheat and 5 mg for ryegrass (resulting in 13C enrichment in the CO2 of the atmosphere of about 44% and 28% 13C, respectively). The 14C 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, CO2 released by root and rhizomicrobial respiration was not flushed out. This allowed CO2 accumulation in the loess—root compartment and the isotopic exchange between respired CO2 and loess CaCO3 by recrystallization. Five days after the labeling, CO2 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 previously assimilated C tracer by roots (≤ 3 d; Kuzyakov and Cheng, 2004) as well as for isotopic exchange between primary CaCO3 and respired CO2 (≤ 4 d; Gocke et al., 2010), and short enough to avoid O2 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 sur-
face (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 CaCO3 by recrystallization, 2 g (corresponding to 70 mg carbonatic C) of every dry loess sample were treated with 15 mL of 3 M H3PO4 in a closed system. Dissolution of samples by acid was chosen instead of combustion in order to release CO2 only from CaCO3 and not from organic compounds (root fragments, microbial remains, exudates). The CO2 evolved from dissolution of CaCO3 was trapped in 12 mL of NaOH to form Na2CO3. As the amount of dissolved CaCO3 was known, an aliquot of the NaOH–Na2CO3 solution was titrated (Zibilske, 1994) to test whether complete CaCO3-C (irrespective if primary or secondary) of the dissolved loess sample was trapped as Na2CO3. This calculation could be applied because in our experiment, formation of secondary CaCO3 in loess did not involve precipitation of additional carbonate but only recrystallization of already present loess CaCO3, as the latter was the sole Ca2+ source.

For δ13C analysis of loess carbonate, trapped CO2 was precipitated as SrCO3 by addition of 0.5 M SrCl2 solution to the NaOH–Na2CO3 solution. No isotopic fractionation took place during precipitation because SrCl2 solution was added in excess and because of the low solubility product of SrCO3 (7 × 10–10). The SrCO3 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. SrCl2 was chosen for precipitation of CO2/C02 instead of commonly used BaCl2, or CaCl2, for the following reasons: Compared to BaCO3, SrCO3 requires lower temperature for thermal decomposition by δ13C analyses on IRMS. At the same time, SrCO3 has much lower solubility product than CaCO3—this ensures an absence of isotopic fractionation by complete precipitation of the dissolved CO2/C02. δ13C from loess CaCO3 under plants and from unlabeled and unplanted loess samples was determined in SrCO3 on an isotope-ratio mass spectrometer (Delta Plus XL IRMS, Thermo Finnigan MAT, Bremen, Germany) connected to an elemental analyzer (EA 3000, Hekatech, Germany). CaCO3 and acetanilide were used as reference materials for δ13C measurement. Results are expressed in permil relative to the V-PDB reference standard, with an absolute precision of > 0.4‰.

To measure 14C incorporated into loess carbonate by recrystallization, dissolution with H3PO4 and trapping of CO2 in NaOH was repeated with 2 g loess (see above), and 14C 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 14C counting efficiency was at least 90%, the measurement error did not exceed 4%. The absolute 14C activity was standardized by the H number method, using a 137Cs external standard.

14C activity of respired CO2 trapped in NaOH was measured on 1 mL aliquots by a liquid scintillation counter (1450 LSC & Luminescence Counter MicroBeta TriLux, Perkin Elmer Inc., USA; 14C-counting efficiency 70%, measurement error ≤ 3.5%) which was standardized by SQP(E). Total carbon content of respired CO2 trapped in NaOH was determined by titration (Zibilske, 1994).

2.3 Calculations

To calculate the amounts of C from respired CO2 incorporated into loess carbonate and the initial rates of secondary carbonate formation, the amount of incorporated C tracer (13C or 14C) was referred to the amount of C tracer in respired CO2-C. The only difference between 13C and 14C approach is that for the former, the atom percent excess (difference be-
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 ($A^{\text{CaCO}_3}_l$) divided by atom percent excess in CO$_2$ respired by $^{13}$C labeled plants ($A^{\text{CO}_2}_l$) and by the time ($t$) between the labeling and the sampling (Eq. 2).

$$A = 100 \cdot \frac{R \cdot \left(\frac{^{13}\text{C}}{^{12}\text{C}} + 1\right)}{1 + R \cdot \left(\frac{^{13}\text{C}}{^{12}\text{C}} + 1\right)}$$

CaCO$_3$ recrystallization rate: $^{13}$C

$$^{13}\text{C} = \frac{A^{\text{CaCO}_3}_l - A^{\text{CaCO}_3}_N}{A^{\text{CO}_2}_l - A^{\text{CO}_2}_N} \cdot t$$

For the second approach, the $^{14}$C specific activity ($^{14}$C$_{\text{SA}}$) 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$_{\text{SA}}$) and total C content ($C_{\text{CO}_2}$) 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 ($C_{\text{CaCO}_3}^{\text{recryst}}$) was calculated using the $^{14}$C activity of loess CaCO$_3$ ($^{14}$C$_{\text{CaCO}_3}^{\text{recryst}}$) (Eq. 4). The amount of recrystallized CaCO$_3$-C was divided by the total CaCO$_3$-C content of the loess ($C_{\text{CaCO}_3}^{\text{CaCO}_3}$) and by the time ($t$) between labeling and sampling (5 d), yielding the initial carbonate recrystallization rate (Eq. 5).

$$^{14}\text{C}_{\text{SA}} = \frac{^{14}\text{C}_{\text{CO}_2}}{C_{\text{CO}_2}}$$

$$C_{\text{CaCO}_3}^{\text{recryst}} = \frac{^{14}\text{C}_{\text{CaCO}_3}^{\text{recryst}}}{^{14}\text{C}_{\text{SA}}}$$

CaCO$_3$ recrystallization rate: $^{14}$C

$$^{14}\text{C} = \frac{C_{\text{CaCO}_3}^{\text{recryst}}}{C_{\text{CaCO}_3}^{\text{CaCO}_3}} \cdot t$$

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.

$^{13}$C values of loess carbonate were ($-1.19 \pm 0.09$)% for unlabeled and unplanted loess, ($-1.06 \pm 0.08$)% for wheat-planted, and ($-1.37 \pm 0.18$)% 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 $6.35 \times 10^{-5}$ d$^{-1}$ (Tab. 1). For ryegrass, amounts of recrystallized CaCO$_3$ and consequently the recrystallization rates, could not be determined because the respective $^{13}$C values were not significantly different from the initial $^{13}$C abundance (Fig. 2a).

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 $2.89 \times 10^{-4}$ d$^{-1}$ and $1.05 \times 10^{-4}$ 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.,
Therefore, the slopes of the trend curves (Fig. 2) correspond to the initial recrystallization rates.

### 3.2 Periods of CaCO₃ recrystallization

Based on the initial rates, periods necessary for complete recrystallization of primary loess carbonate were calculated. Assuming that not only the primary loess CaCO₃, but also secondary CaCO₃ is recrystallized with CO₂ released by root and rhizomicrobial respiration, the increase of the amount of recrystallized carbonate is described by an exponential approach (Eq. 6). As high CO₂ 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 (CaCO₃(t)) was calculated as follows:

\[
CaCO₃(t) = 100 \cdot \left(1 - \exp\left[-t \cdot \frac{\text{rate}}{365}\right]\right),
\]

with \(t\): time in years, \(\text{rate}\): recrystallization rate in d⁻¹, \(\text{GS}\): growing season in days per year.

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

### 4 Discussion

#### 4.1 Isotopic pulse labeling

Based on the exchange of primary loess CaCO₃-C with CO₂ from root and rhizomicrobial respiration, we used the isotopic exchange to estimate the amount of recrystallized CaCO₃ in loess. \(^{13}\text{C}\) and \(^{14}\text{C}\) isotopes were employed simultaneously as tracers to test their feasibility for assessing the very slow carbonate recrystallization process.

Due to increased CO₂ partial pressure (CO₂ 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 \(^{14}\text{C}\) specific activity of CO₂ released by root and rhizomicrobial respiration, which in turn was used to calculate the recrystallization rate based on the \(^{14}\text{C}\) 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 1: CaCO₃ recrystallization rates in rhizosphere and nonrhizosphere (only \(^{14}\text{C}\)) loess calculated based on \(^{13}\text{C}\) and \(^{14}\text{C}\) labeling, derived from loess planted with wheat and ryegrass. For ryegrass, the \(^{13}\text{C}\) approach did not provide reasonable results, which is also reflected in Fig. 2. For comparison, ranges of recrystallization rates without plants under CO₂ concentrations between 380 and 50 000 ppm in loess air (Gocke et al., 2010b) are also displayed.

<table>
<thead>
<tr>
<th>Isotopic approach</th>
<th>Wheat rhizosphere (nonrhizosphere)</th>
<th>Ryegrass rhizosphere (nonrhizosphere)</th>
<th>Without plants (Gocke et al., 2010b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{13}\text{C})</td>
<td>6.35 [± 4.00] × 10⁻⁵</td>
<td>–3.77 [± 3.74] × 10⁻⁴</td>
<td>0.3 × 10⁻⁵ ... 1.4 × 10⁻⁵</td>
</tr>
<tr>
<td>(^{14}\text{C})</td>
<td>2.89 [± 0.13] × 10⁻⁴</td>
<td>1.05 [± 0.06] × 10⁻⁴</td>
<td>0.4 × 10⁻⁶ ... 1.7 × 10⁻⁶</td>
</tr>
</tbody>
</table>

#### Table 2: Periods necessary for 99% recrystallization of rhizosphere-loess CaCO₃, calculated based on \(^{13}\text{C}\) and \(^{14}\text{C}\) 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.

<table>
<thead>
<tr>
<th>Isotopic approach</th>
<th>Wheat rhizosphere</th>
<th>Ryegrass rhizosphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{13}\text{C})</td>
<td>590 [365–1600]</td>
<td>n.d.*</td>
</tr>
<tr>
<td>(^{14}\text{C})</td>
<td>130 [125–140]</td>
<td>240 [225–255]</td>
</tr>
</tbody>
</table>

* n.d. not determined
4.2 Estimated CaCO₃ 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 CO₂ supply during the initial stage (weeks to months) of plant growth leads to linear increase of recrystallized CaCO₃. Thus, slopes of Fig. 2 correspond to initial recrystallization rates in loess and can be used as approximate values for comparison of ¹³C and ¹⁴C results.

The results based on ¹⁴C labeling showed that the methodological sensitivity of the ¹⁴C approach is high enough to detect process rates as slow as CaCO₃ recrystallization in plant experiments. The ¹⁴C 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 ¹³C approach produced usable results only for wheat (6.35 × 10⁻⁵ d⁻¹). For ryegrass, no accumulation of ¹³C in loess CaCO₃ 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 (¹³C vs. ¹⁴C 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 ¹³C (up to ± 100% of the mean) compared to that based on ¹⁴C (max. ± 6% of the mean, Fig. 2, Tab. 1).

4.3 Precision of ¹³C and ¹⁴C approaches

The recrystallization rate based on ¹³C incorporation in CaCO₃ in the loess close to the root surface (rhizosphere) was one order of magnitude lower than rates based on ¹⁴C incorporation and showed much higher standard errors (Tab. 1). Therefore we did not analyze δ¹³C in nonrhizosphere loess carbonate because we assumed even less reliable values there. In contrast, the ¹⁴C 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 ¹³C 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 ¹³C mass spectrometry is 6 orders of magnitude less (10⁻⁷ mol) than that of ¹⁴C liquid scintillation counting (10⁻¹³ mol). Second, in case of ¹³C labeling, the ¹³C is already present in CaCO₃ of unplanted and unlabeled loess. Although the ¹³C content is increased by labeling loess carbonate, the amount of ¹³C incorporated remains very small due to the very low recrystallization rates (even after periods longer than in our study). Therefore, the amount of ¹³C incorporated in the carbonate is still extremely low compared to that already present in loess. Accordingly, analyses of δ¹³C near the level of natural abundance depend strongly on measurement accuracy. This problem does not exist in ¹⁴C 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 ¹⁴C content in the loess CaCO₃ is therefore zero.

Despite careful sampling and sample preparation (mixing of loess samples, dissolution of CaCO₃, reprecipitation as SrCO₃, washing, and centrifugation), a variation of 1%–2% between replications of the same treatment can occur due to inhomogeneous distribution of ¹³C incorporated into CaCO₃. Because of high δ¹³C 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 CaCO₃-δ¹³C values based on ¹⁴C data (Tab. 1 and Fig. 2b) yielded very small changes of the initial δ¹³C of loess CaCO₃ (0.24‰ for wheat and 0.04‰ for ryegrass). These changes are too low for reliable ¹³C analysis. Therefore, we strongly recommend application of ¹⁴C tracer for estimation of initial CaCO₃ recrystallization rates. Accordingly, the isotopic exchange based on ¹⁴C is probably the only possibility to estimate such slow process rates.

As shown in this study, the recrystallization rate based on the ¹³C approach could be calculated only for wheat plants, which received twice as much ¹³C (10 mg per plant pot) as ryegrass plants (5 mg per plant pot). One potential way to bypass the low sensitivity of ¹³C labeling might be to increase the amounts of ¹³C applied, boosting the percentage of ¹³C applied for the pulse, thus leading to a higher percentage of ¹³C recovered in secondary carbonate. This, however, might entail methodological difficulties (overpressure in the labeling chamber by the high amount of released CO₂, potentially incomplete assimilation by plants because of CO₂ oversupply). It might also lead to unnatural partitioning of assimilates due to very high CO₂ content in the chamber. In contrast, the CO₂ concentration in the chamber is increased only marginally when applying ¹⁴C because the mass of ¹⁴C necessary to estimate the recrystallization rate is negligibly low (µg).

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

Compared with literature data (Kuzyakov et al., 2006), the ¹⁴C approach showed high reproducibility of rates (10⁻⁵–10⁻⁴ d⁻¹ for nonrhizosphere loess, 10⁻⁴ d⁻¹ for rhizosphere loess). In the ¹³C approach, lower sensitivity, high standard errors of means between replications 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. ¹⁴C isotopic exchange clearly yields more dependable results.

One further indicator of the better reliability of ¹⁴C over ¹³C is the fact that, without plants, the rates calculated based on...
13C (10^−5 d−1) were higher than when using 14C (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 13C and 10^−4 d−1 for 14C) (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 13C 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 CO2 (Fischer et al., 2010). Moreover, the CO2 evolved from root and rhizomicrobial respiration and dissolved as HCO3− (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 (δ13C) or by 14C 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 14C than by 13C 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 13C labeling, 590 y were necessary for 99% recrystallization of primary loess carbonate, while the 14C 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 13C-based rate (Tab. 1), however, the 99% recrystallization period based on 13C data varies between 365 and 1600 y (Tab. 2). The 14C 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 rye-grass, 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 14C age of CaCO3 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 CaCO3 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 14C 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 14C 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 14C 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., 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 CO2 concentrations in loess due to sealing of the plant pots probably led to enhanced dissolution of loess CaCO3 and precipitation of secondary CaCO3, 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 14C approach better fit with radiocarbon ages measured on carbonate materials from soil profiles of known ages.
5 Conclusions

Assessing very slow CaCO₃ 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 CO₂, we calculated initial rates by determining the amount of C incorporated into secondary carbonate from respired CO₂ of dual ¹³C and ¹⁴C pulse–labeled plants.

We showed that very small portions of primary loess carbonate were recrystallized in the rhizosphere, leading to rates of \(10^{-5} \text{ d}^{-1}\) (¹³C approach) and \(10^{-4} \text{ d}^{-1}\) (¹⁴C approach). Extrapolating the rate estimated by ¹³C 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 ¹⁴C labeling showed sufficiently higher precision and reproducibility and indicated full recrystallization periods of 130 (125–140) or 240 (225–255) y. Therefore, the ¹⁴C 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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CaCO3 recrystallization assessed by 13C and 14C 817


