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VIEWPOINT

Invited Contribution for the 2022 Anniversary Edition

Biogeochemical limitations of carbon stabilization in forest subsoils[#]

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Abstract

Background: Soils are important carbon (C) sinks or sources and thus of utmost importance for global carbon cycling. Particularly, subsoils are considered to have a high potential for additional C storage due to mineral surfaces still available for sorptive stabilization.

Aims: Little information exists about the extent to which additional litter-derived C is transferred to and stabilized in subsoils. This study aimed at evaluating the role of litter-derived dissolved organic matter (DOM) inputs for the formation of stable mineral-associated C in subsoils.

Methods: We carried out a multiple-method approach including field labeling with ^{13}C-enriched litter, exposure of ^{13}C-loaded reactive minerals to top- and subsoils, and laboratory sorption experiments.

Results: For temperate forest soils, we found that the laboratory-based C sink capacity of subsoils is unlikely to be reached under field conditions. Surface C inputs via litter leachates are little conducive to the subsoil C pool. Only 0.5% of litter-derived C entered the subsoil as DOM within nearly 2 years and most of the recently sorbed C is prone to fast microbial mineralization rather than long-term mineral retention. Desorption to the soil solution and an adapted microbial community re-mobilize organic matter in subsoils faster than considered so far.

Conclusions: We conclude that the factors controlling the current mineral retention and stabilization of C within temperate forest subsoils will likewise limit additional C uptake. Thus, in contrast to their widely debated potential to accrue more C, the role of forest subsoils as future C sink is likely overestimated and needs further reconsideration.

KEYWORDS
carbon cycling, climate change mitigation, microbial community composition, mineral-associated organic carbon

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1 | INTRODUCTION

Globally, 50% of soil organic carbon (SOC) is stored in the subsoil below 30 cm depth, making subsoils one of the largest terrestrial C reservoirs (Ciais et al., 2013; Schimel, 1995). Recently, fixed C is usually transferred to subsoils via dissolved organic matter (DOM) or inputs of roots (Rumpel et al., 2012). A paramount relevance of DOM and its sorption to minerals for SOC accumulation are advocated by the oftentimes negligible contents of root residues and high ^{14}C ages of organic matter in subsoils (Torn et al., 1997). The extent to which soils can accumulate organic C (OC) thus largely depends on available sorption sites at reactive minerals, for example, pedogenic metal (oxyhydr)oxides (Hagedorn et al., 2015; Kramer & Chadwick, 2018; Rasmussen et al., 2018) and clay minerals (Rasmussen et al., 2018), and is limited by their sorption capacity (Guggenberger & Kaiser, 2003) or by reaching steady-state equilibrium (Sommer & Bossio, 2014).

While most aboveground C inputs are retained in mineral topsoil horizons (Fröberg et al., 2007), subsoils contain considerable amounts of uncovered mineral surfaces (Guggenberger & Kaiser, 2003). Based on sorption experiments in the laboratory (Abramoff et al., 2021; Mayes et al., 2012) or on models (Robertson et al., 2019), forest subsoils are assumed to have an enormous capability to retain additional C (Lorenz et al., 2011; Mayes et al., 2012; Rumpel et al., 2012). Consequently, forest ecosystems are thought to act as future C sinks if managed toward an enhanced C input to soil (Kaipainen et al., 2004; Lal, 2004; Luyssaert et al., 2008). A human-induced greening of the vegetation has been observed globally (Zhu et al., 2016) and of the northern hemisphere in particular (Mao et al., 2016) since the 1980s, indicating more productive forests with presumably increasing C inputs into the soils (Pan et al., 2011). The observed greening is particularly related to the growth of forests <140 years (N. G. McDowell et al., 2020) and enhanced net primary production (Liu et al., 2015), and is predominantly connected to forest management, nitrogen deposition, CO_2 fertilization, and climate change (Magnani et al., 2007; Mao et al., 2016; Zhu et al., 2016). But many forests, including widespread temperate forests, are located in high sensitivity biomes (Kramer & Chadwick, 2018), which can vastly react to changing environmental conditions. For example, disturbances as droughts and windthrow events can additionally contribute to temporary enhanced C inputs to soil by dead biomass (Anderegg et al., 2020; N. G. McDowell et al., 2020). Field experiments, however, suggest that increased aboveground litter inputs have little or no effect on soil C stocks (Lajtha et al., 2018). Not only that most of this additional input is rapidly mineralized (Lajtha et al., 2018), it is also thought to foster C mobilization in mineral topsoils and its translocation toward deeper subsoils and aquifers (Guggenberger & Kaiser, 2003; Kaiser & Kalbitz, 2012). Carbon released to soil by rhizodeposition may be efficiently stabilized in soil (Sokol & Bradford, 2019), but has been also suggested to trigger C liberation (Keiluweit et al., 2015). Given the considerable C inputs to subsoils (Kalbitz & Kaiser, 2008; Sokol & Bradford, 2019) but overall small and old SOC stocks (Rumpel et al., 2012) raises concerns whether the postulated potential for further subsoil C storage at mineral surfaces (Abramoff et al., 2021; Mayes et al., 2012) can be exploited under field conditions.

Our central hypothesis was that under current steady-state conditions, C inputs into subsoils are largely reversibly bound by minerals and do not promote the formation of long-term stable SOC. This "staying-below-capacity" effect could substantially reduce the value of forest subsoils for additional C sequestration and challenges the perception that forest subsoils can store more C in associations with minerals than they currently do. We further hypothesized that the existing biogeochemical limitations in the subsoil C storage likewise apply to scenarios of raising C inputs and thus interfere in exploiting the C storage potential of subsoils.

These hypotheses were tested by multiple-method approach based on field and laboratory experiments. A 20-m^2 field labeling experiment with ^{13}C-enriched litter (Liebmann et al., 2020) with a unique long-term DO^{13}C and $^{13}CO_2$ monitoring (Leinemann et al., 2016; Wordell-Dietrich et al., 2020) was used to test whether recent litter layer inputs to the subsoil are readily available to microbial consumption and contribute little to subsoil C stocks. We further employed laboratory batch sorption experiments to determine the theoretical capacity for additional DOC sorption. Lastly, in an in situ C exchange field experiment with C-coated minerals, we evaluated the extent to which mineral-associated C is susceptible to desorption and microbial utilization under current subsoil conditions.

2 | MATERIALS AND METHODS

2.1 | Study sites

The monitoring and main labeling experiments were carried out in the Grinderwald beech forest (*Fagus sylvatica* L.) 40 km north-west of Hanover, Germany (52°34'22'' N, 9°18'49'' E). The beech forest was established in 1916 and replaced a former pine forest. Mean annual temperature is 9.7°C, and mean annual precipitation accounts to 762 mm (Deutscher Wetterdienst, period 1981–2010). Soils are primarily Dystric Cambisols, which developed on Pleistocene glacio-fluvial sandy deposits (IUSS Working Group WRB, 2015). Soil texture is dominated by sand-sized particles. For a more detailed site description, please refer to Bachmann et al. (2016) and Angst, Kögel-Knabner et al. (2016). The laboratory batch experiments included two additional sites under beech, Ebergötzen (Dystric Cambisols) and Rüdershausen (Haplic Luvisols), as did the related DOC injection experiment from Kalks et al. (2020) in order to investigate the sorption behavior of soils which developed on different parent materials (more details are provided in the Supporting Information Methods). For the three study sites, we refer to a soil depth between 0 and 10 cm as topsoil, 10 and 50 cm as upper subsoil, and 50 and 150 cm as deeper subsoil based on recent publications describing these sites (Kalks et al., 2020; Leinemann et al., 2016; Liebmann et al., 2020).

2.2 | Litter manipulation experiment and flux monitoring

Three subsoil monitoring observatories (distance about 60 m) were established in 2013 in Grinderwald (Leinemann et al., 2016). They are equipped with multi-segment suction plates for soil solution sampling (25 × 25 cm, ecoTech Umwelt-Meßsysteme GmbH, Bonn, Germany), with 16 squared segments (36 cm^2) each, installed at 10, 50, and 150 cm soil depth (Leinemann et al., 2016), suction cups, and CO$_2$ sensors at 10, 30, 50, and 90 cm soil depth for analysis of soil CO$_2$ concentration profiles in combination with closed chambers for measurement of total soil CO$_2$ efflux (Wordell-Dietrich et al., 2020) (Figure S1A). A field labeling approach was performed on the 13.2 m^2 circular catchment area of each of the three monitoring observatories by replacing the natural litter layer on 50% (i.e., 6.6 m^2 per observatory) of the area with ^{13}C-enriched litter (Figure S1B), the other 50% remained unlabeled. Taken all observatories together, an area of about 20 m^2 was prepared with the labeled beech litter. Initial ^{13}C enrichment varied between 1241‰ and 1880‰ for the three observatories and in total, about 275 g labeled litter per m^2 was applied, which simulated an average annual litter fall in German beech forests (Meier et al., 2005). More details on the labeling approach are provided in the Supporting Information Methods. Sampling of soil solution and soil air (Wordell-Dietrich et al., 2020) was done on a weekly basis between January 2015 and November 2016, and DOC and CO$_2$ and their δ^{13}C values were determined. After 22 months, the remaining labeled litter was removed and soil cores down to 200 cm soil depth were taken to account for the incorporation of labeled litter-derived C in SOC and functional organic matter fractions, including mineral-associated OC (MAOC) and particulate OC (POC), separated by density fractionation (Liebmann et al., 2020) (more details are provided in the Supporting Information Methods).

2.3 | Laboratory batch sorption and desorption experiments

Batch sorption and desorption experiments were conducted with soil samples from the three beech (*F. sylvatica* L.) forest sites Grinderwald, Rüdershausen, and Ebergötzen in Lower Saxony (Germany). Soils developed on different parent materials, including sandy glacio-fluvial, loess deposits, and red sandstone. Sorption isotherms were recorded with bulk soil samples from 10, 50, and 100 cm soil depths and DOC extracts from local beech litter in eight different concentrations up to 400 mg C L^{-1}. For that, mixtures with a soil:solution ratio of 1:5 were shaken horizontally for 24 h at a frequency of 1.7 s^{-1}, the remaining DOC was determined in the supernatant, and the amount of sorbed C was calculated by difference of the initial and final DOC concentration (Kaiser et al., 1996). Gross OC sorption and exchange with native OC was quantified with a DOC extract from ^{13}C-enriched beech litter applied at the highest initial DOC concentration of the sorption isotherm. Subsequent desorption experiments were carried out under the same conditions as the sorption experiments by adding an ionic background (IB) solution to the soil (Saidy et al., 2013), which was prepared on the basis of the IB of the DOM solutions and included the following anions and cations: Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, Na^+, K^+, Mg^{2+}, and Ca^{2+} (more details are provided in the Supporting Information Methods).

2.4 | In situ MAOC exchange experiment

The field experiment for the in situ C exchange on mineral surfaces was conducted on an adjacent plot of the soil monitoring study site Grinderwald, following a previous laboratory approach (Leinemann et al., 2018). Goethite and vermiculite, representative for two groups of pedogenic minerals responsible for the majority of reactive surfaces in temperate soils, were coated by sorption of DOM extracted from ^{13}C-enriched beech litter. The minerals had C loadings of 4–9 mg C g^{-1} mineral with δ^{13}C ratios of 731‰–1014‰ and were buried in mesh bags with a 20-μm mesh size in triplicates in 10, 50, and 150 cm soil depth. After 24 months of field exposure, mesh bags were removed and analyzed for bulk C, δ^{13}C, and the microbial community composition (more details are provided in the Supporting Information Methods).

3 | RESULTS AND DISCUSSION

3.1 | Translocation of litter-derived carbon toward forest subsoils

The application of ^{13}C-enriched litter on the surface of subsoil observatories at the central study site Grinderwald (Liebmann et al., 2020) allowed us to trace fluxes of litter-derived C into different C pools and to create a 22-month C balance at various soil depths. For that, we combined time-integrated DOC and CO$_2$ fluxes and directly measured pools including MAOC, POC, and aboveground litter. While CO$_2$ and MAOC, POC data were adopted from Wordell-Dietrich et al. (2020) and Liebmann et al. (2020), respectively, incorporation of new litter and DOC data resulted in a novel litter-derived C budget down to the deep mineral subsoil. We were able to recover about 84% of the initially applied tracer. More than one-third of the applied litter C was directly mineralized and lost to the atmosphere, while another third remained at the surface after nearly 2 years (Figures 1 and S2). About 9% particulate-litter C was translocated into the mineral topsoil. Only 1.5% of the litter C was mobilized as DOC and immobilized as MAOC in the topsoil horizon (0–10 cm soil depth). Soil solutions were collected with segmented suction plates, covering for the first time both, DOC fluxes through matric and preferential flow path domains (Leinemann et al., 2016) (the Supporting Information Methods). About 0.5% of the ^{13}C-labeled litter C entered the upper subsoil in a depth >10 cm via the DOC pathway (Figure 1). In addition, only 0.003% labeled litter-derived C was incorporated into the total microbial biomass in the soil profile, particularly in the topsoil (Preusser et al., 2021). These results show that most of the litter inputs are retained in the topsoil, and the majority of the DOC in the

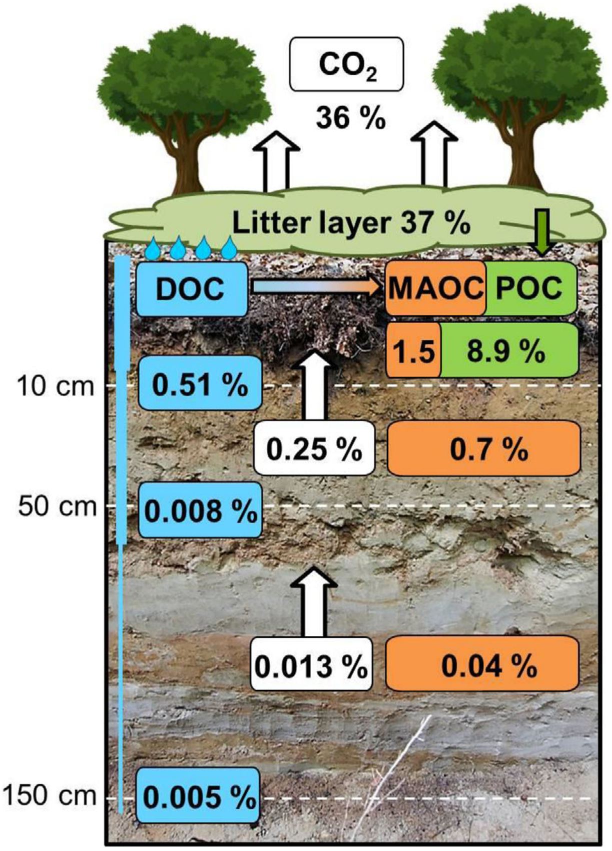

FIGURE 1 Recovery of litter-derived carbon (C) into different C pools in topsoil (10 cm), upper subsoil (50 cm), and deeper subsoil (150 cm) over the course of 22 months in a Dystric Cambisol located in a temperate European beech forest in Germany. Values are given in percent of the initially applied labeled litter. Evaluated C pools included the CO_2 production at the soil surface and in the depth increments 10–50 and 50–90 cm (white), leachates of dissolved organic C (DOC) in 10, 50, and 150 cm soil depth (blue), and incorporation of litter material in the depth increments 0–10, 10–50, and 50–150 cm, due to bioturbations as C in particulate organic matter (POC; green) and due to sorption to mineral surfaces thereby forming mineral-associated organic C (MAOC; brown). Residual litter after the field exposure was quantified and is given in the litter layer. The complete C balance revealed a recovery of initially applied litter of about 84%. CO_2 data were adopted from Wordell-Dietrich et al. (2020), and MAOC/POC differentiation was adopted from Liebmann et al. (2020). The latter authors did not analyze the MAOC in the depth increment 50–100 cm, but reported an interpolation of 0.03% which we added here

mineral top- and subsoil originate from older native SOC (Figure S3). Our findings support previous findings (e.g., Fröberg et al., 2009, 2007; Hagedorn et al., 2004), but at the same time complement them by including the overlooked deep mineral subsoil >100 cm depth. It took about 1 year after ^{13}C-litter application before small amounts of litter-derived DOC showed up in the subsoil solution (Figure S4), indicating a significant lag-phase likely due to multiple sorption-decomposition-desorption cycles through the soil matrix (Kaiser & Kalbitz, 2012). This was substantially slower than infiltrating water after strong precipitation events, reaching the deep subsoil (150 cm) in a few weeks only (Figure S5).

About 0.7% of the applied litter C was found as MAOC in the upper part of the subsoil (10–50 cm depth) after 22 months (Figure 1), which matches the range of litter-derived $DO^{13}C$ inputs into the upper subsoil, that is, 0.5% of added litter C. The ratio of litter-derived CO_2 production in the upper subsoil (0.25% of the added litter C) and retained C (0.25 + 0.7 = 0.95%) shows that about one-fourth of the litter layer inputs entering the upper subsoil were respired within less than 2 years (Figure 1). In the deep subsoil, this ratio remained constant, indicating that substantial parts of fresh C inputs are not stabilized over longer periods (Liebmann et al., 2020) but rather represent a labile C source (Schrumpf et al., 2013), independent of soil depth and potentially available mineral surfaces. In fact, litter-derived CO_2 was observed in all soil depths right from the start of the experiment (Figure S6), suggesting rapid mineralization of recent organic compounds without or with only very short temporal immobilization by the mineral phase (Kaiser & Kalbitz, 2012). In accordance, high apparent ^{14}C ages of subsoil SOC indicate that rejuvenation by fresh inputs is negligible (Rumpel et al., 2012). The microbial utilization of fresh DOC inputs is also reflected by the overall high contribution of microbial-derived carbohydrates in subsoil soil solutions (Gunina & Kuzyakov, 2015; Kaiser & Kalbitz, 2012; Roth et al., 2019) (Table S1, Figure S7). We conclude that recent litter-derived DOC facilitates predominantly MAOC formation in the topsoil, with only limited supply of C to subsoils.

3.2 | Subsoil capacities for additional carbon uptake

Even though recent litter contributes little to soil DOC fluxes (e.g., Fröberg et al., 2007), considerable amounts of DOC are percolating to the subsoil at the Grinderwald study site, which are in the range of DOC fluxes reported for other sandy soils in temperate ecosystems (Fröberg et al., 2006; W. H. McDowell & Likens, 1988; Michalzik et al., 2001). The total DOC flux in 150 cm soil depth amounts to 1.8 g m^{-2} y^{-1} (Table S1), which corresponds to an annual DOC input of more than 2% of the mean native SOC stock (88 g m^{-2} in 140–160 cm). Therefore, we explored the sorption capacities of forest top- and subsoils and the vulnerability of sorbed ^{13}C-labeled organic matter toward desorption in batch experiments. To assess the sorption capacity, we used three temperate European beech forest soils with a typical clay mineral assemblage (dominated by vermiculite, chlorite, illitic clay, and kaolinite; Figure S8), but varying in soil texture (silty to sandy). Sorption isotherms showed that typical topsoil DOC concentrations of 50–100 mg L^{-1} (Leinemann et al., 2016) (Table S1) induced desorption of native SOC from topsoils (Figure 2), supporting that topsoils already reached their steady state (Mikutta et al., 2019). Similarly, typical subsoil DOC concentrations of <10 mg L^{-1} (Fröberg et al., 2007; Leinemann et al., 2016) (Figure 2; Table S1) resulted either in no additional net C uptake or in a net release of native SOC from subsoils (Figures 2 and S9). Like observed under field conditions (Kaiser et al., 1996; Leinemann et al., 2016), a decrease in specific ultraviolet (UV) absorption at 280 nm with

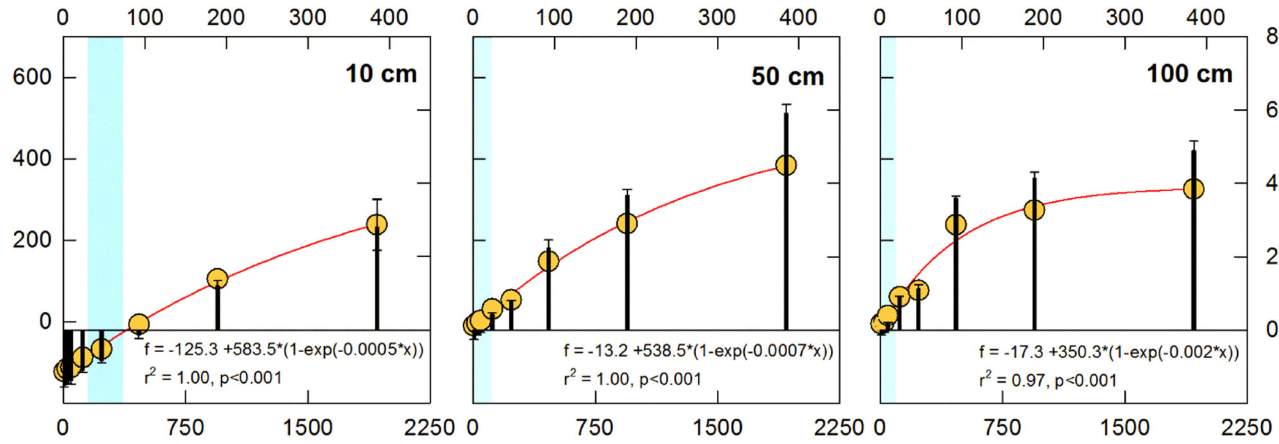

FIGURE 2 Dissolved organic C (DOC) sorption isotherms of soil from 10, 50, and 150 cm soil depth of a Dystric Cambisol located in a temperate European beech forest in Germany. Both *x*-axes show the DOC added to the soil, on the bottom *x*-axis normalized to soil mass, on the top *x*-axis as concentration. Sorption isotherms (yellow circles and red line) show the released or sorbed DOC after the experiment (left *y*-axis, $n = 3$) as a function of added DOC. Black bars show the quantified DOC sorption (right *y*-axis, $n = 3$), estimated for 1 m^2 and a soil thickness of 10 cm (since soil samples for the sorption experiments were taken as composite samples of a 10 cm increment; 10–20, 50–60, 100–110 cm). The blue-marked areas represent typical DOC concentrations (top *x*-axis) in the respective soil depths measured at this study site (see Table S1). Concentrations were calculated as the mean of all samples taken during the timeframe February 2015 to November 2016, and amounted to 51.5 ± 22.0 mg L^{-1} in 10 cm ($n = 112$), 13.0 ± 9.0 in 50 cm ($n = 60$), and 8.6 ± 11.2 in 150 cm ($n = 88$). The DOC concentration in 150 cm soil depth was used for the 100 cm variant in this figure

soil depth indicated preferential sorption of aromatic compounds and mobilization of native organic matter of lower aromaticity (Kaiser & Kalbitz, 2012) (Figure S10). It was thus unexpected that the addition of a low concentrated but still aromatic-rich beech litter extract, corresponding to an input of about 11% of the native SOC stock, induced exchange/desorption of C instead of exploiting the available mineral sorption sites. Subsoils were only able to retain more C at substantially higher DOC concentrations of >>10 mg L^{-1}, showing their high sorption capacity under laboratory conditions. These results suggest further C uptake only for highly sorptive DOM and DOC concentrations considerably higher than under present field conditions. But natural subsoil solutions are usually dominated by much less reactive organic compounds such as carbohydrates (Kaiser & Kalbitz, 2012) (Figure S7). We infer that a disadvantage of the past and current adsorption experiments is the use of highly reactive litter-derived DOM as standard, whereas the inherent DOM composition of solutions entering a particular soil depth would be more representative for field situations. It must be further noted that, unlike in laboratory sorption experiments, the sorption capacity of natural subsoils may not be reached as DOM percolates also along preferential flow paths (Leinemann et al., 2016), thus bypassing possible sorption sites.

Previous studies with pristine pedogenic minerals suggest a strong sorption-desorption hysteresis of freshly retained C and a subsequent decrease in mineralization rates (Mikutta et al., 2007; Saidy et al., 2013). Our batch desorption experiment with natural soils indicates that newly sorbed litter-derived C was held in weaker associations. Thus, a significant part remained mobilizable shortly after sorption (20–39% of sorbed C, $p < 0.05$) in the deeper subsoil samples, whereas older, native C was more strongly bound (Figures S11 and S12). Recent investigations into in situ mineral-organic associations of various soil depths likewise revealed short retention times of newly sorbed C (Liebmann et al., 2020). As sorption-desorption processes are governed by the in situ solution equilibrium and sorption site availability differs between field and laboratory conditions, the laboratory-based C sorption capacity of forest subsoils remains an unsuitable predictor for further C sequestration.

3.3 | Soil minerals and carbon sequestration

In the previous sections, we showed that considerable DOC fluxes and available sorption sites in forest subsoils contrast their low C stocks. This discrepancy may be explained by the fact that newly formed MAOC in subsoils undergoes frequent mobilization, thus maintaining the current equilibrium state. Therefore, we further tested the persistence of subsoil MAOC under in situ field conditions. Reactive soil minerals (goethite and vermiculite) coated with ^{13}C-enriched organic matter, with C contents similar to those of the subsoil clay fraction (Angst, Kögel-Knabner et al., 2016), were buried in the soil profile. We expected a slow in situ C cycling in the subsoil with small rates of mobilization, which is prerequisite for further C sequestration. After a 2-year exposure period, the gross C balance revealed a high exchange rate of C in the topsoil (Figure 3; Table S2), which can be ascribed to the regular input of fresh organic compounds (Table S1) and an active microbial community colonizing the minerals (Figure S14). Goethite acted as net C sink in the topsoil as its sorption capacity was still not reached (Kaiser & Guggenberger, 2007) and due to the presence of highly reactive aromatic DOC constituents (Figure S7, Table S1). In the subsoil, however, both minerals were net C sources.

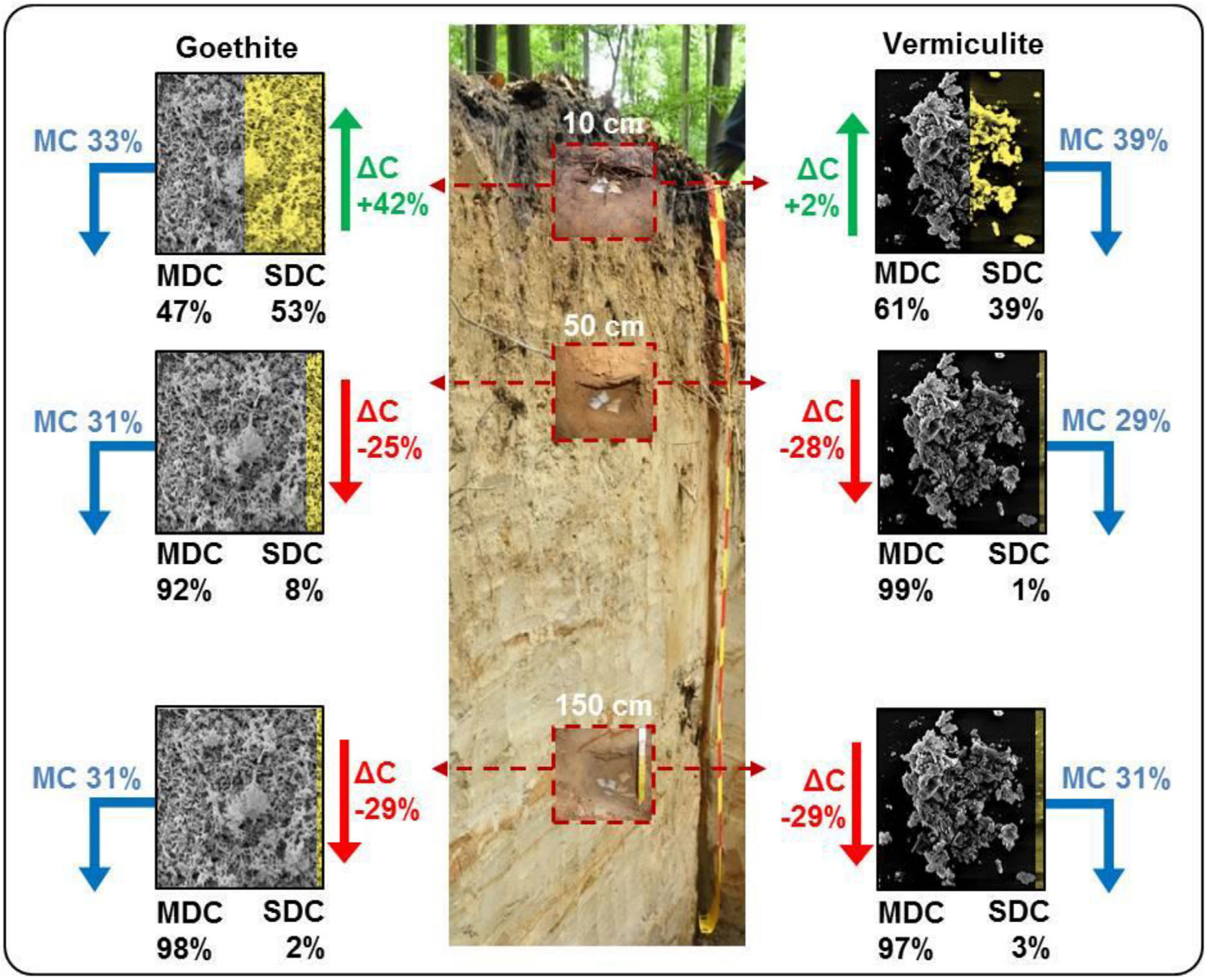

FIGURE 3 Depth-dependent gross carbon (C) exchange of ^{13}C-labeled mineral-associated organic C (MAOC) after 2-years of field exposure in a Dystric Cambisol located in a temperate European beech forest in Germany. Goethite was chosen as a representative pedogenic Fe oxyhydroxide (left side) and vermiculite as representative 2:1 clay mineral (right side). The net difference in C content before and after the field exposure is given in percent of the initial MAOC content (ΔC). The amount of mobilizable C (MC) during the field exposure is given in percent of initial MAOC as well. The final C content after field exposure was differentiated in pre-existent ^{13}C-labeled mineral-derived C (MDC) and fresh unlabeled solution-derived C (SDC; yellow color). The soil profile in the center representatively shows a Dystric Cambisol in the Grinderwald forest (Germany); left and right figures show scanning electron microscope (SEM) images of the respective C-loaded minerals

Carbon exchange and microbial-driven C cycling on mineral surfaces in the topsoil support the assumption that mineral surfaces in topsoils are the place of pronounced C cycling (Kaiser & Kalbitz, 2012). However, minerals buried in the subsoil exposed to natural subsoil DOC with small concentrations showed a net release of C without concomitant retention of fresh C from the soil solution (Figure 2, Table S1). A mobilization of 30–40% of native C from both minerals (Figure 3) suggests a mobilization process independent of the mineral composition and, contrary to expectations, also independent of soil depth. The minerals loaded with litter-derived C represent microbial hotspots (Kandeler et al., 2019; Kuzyakov & Blagodatskaya, 2015) and thus promoted the growth of bacteria compared to the surrounding bulk soil, especially of copiotrophic Betaproteobacteria (Leinemann et al., 2018) (Figure S13). Their activity might be further fueled by the carbohydrate-rich DOM, as with increasing soil depth, the soil solution comprises an increasing proportion of carbohydrates (Figure S7) with a low affinity to form bonds to mineral surfaces (Kalbitz et al., 2005). Additional C input may thus promote the growth of copiotrophic microorganisms, which benefit most from easily available C sources (Leinemann et al., 2018). The observed high rate of MAOC mobilization in the subsoil is consistent with field findings from the same site, showing a loss of about two-third of fresh mineral-bound C within 18 months (Liebmann et al., 2020). Similarly, our batch sorption-desorption experiments with native soil samples and DO^{13}C solution further indicate that there is a frequent exchange of DOC with native SOC and a preferential desorption of recently retained C, irrespective of the mineral composition and being more pronounced in subsoil samples compared to topsoil samples (Figure S12). Overall, the in situ field exposure experiment suggests that the mineral C storage ability in subsoil environments is limited. Besides depending on mineralogical properties and subsoil DOC concentrations, our data emphasize the active role of an adapted microbial community associated with such pedogenic minerals for C remobilization (Leinemann et al., 2018).

4 | IMPLICATIONS FOR FOREST SUBSOILS AS FUTURE CARBON SINKS

Subsoils are highly discussed regarding their potential to sequester C (Abramoff et al., 2021; Fontaine et al., 2007; Sommer & Bossio, 2014), thereby helping to counteract the globally increasing atmospheric CO_2 concentrations. Our integrative approach of field labeling with flux monitoring, laboratory sorption experiments, and in situ exposure of C-coated minerals suggests that under field conditions, the mineral-controlled potential of temperate forest subsoils to serve as a future C sink may be largely overestimated. Our study unraveled factors that explain the discrepancy between the theoretical C storage capacity of subsoils, as determined in laboratory experiments, and the C sequestration realized under natural conditions. We identified several factors that supposedly limited C accrual in the past and likely also prevent significant future C sequestration in temperate subsoils with potentially increasing standing biomass, contradictory to the proposed role of subsoils as additional C sinks: (1) Dissolved organic C inputs into subsoils, which potentially bind to mineral surfaces, are too small to induce substantial additional sorption compared to batch experiments, rather dominated by less sorptive carbohydrate-rich organic matter (Kaiser & Kalbitz, 2012), and cannot reach all mineral sorption sites as in laboratory experiments due to the structural heterogeneity of soils (Hagedorn et al., 2015). (2) As recent forest subsoils are in equilibrium between inputs and outputs of C, a further C accumulation would require substantially enhanced inputs of highly sorptive plant-derived organic compounds to subsoils over long periods. However, this study along with previous investigations (Fröberg et al., 2007; Kaiser & Kalbitz, 2012; Liebmann et al., 2020) clearly revealed that only a very limited amount of litter-derived organic matter can enter the subsoil. Besides the option to increase aboveground inputs, the adjustment of forest management with the aim to increase belowground inputs via more rhizodeposits is considered a promising measure (Lorenz et al., 2011). But subsoil plant residues consisting of dead fine roots were also found to turn over rather fast within 20 years (Angst, John et al., 2016; Gaul et al., 2009), while metabolites of this root decay then potentially contribute to MAOC. Although the formation of mineral-organic associations is key for the build-up of C stocks, we showed that C inputs into subsoils consistently lead to weaker mineral bindings and thus not to a persistence of newly formed MAOC. This is consistent with the observation that increasing C loadings of minerals cause less stable mineral-organic bindings (Guggenberger & Kaiser, 2003; Kaiser & Guggenberger, 2007). In this respect, we consider (3) that increased C input into forest subsoils promotes the formation of rather labile MAOC and microbial mobilization of the inherent OC. Additional inputs of labile C may thus even unlock the old native carbon pool by co-metabolic decomposition (Fontaine et al., 2007) or direct dissolution of mineral sorption partners by root exudates (Keiluweit et al., 2015). Recently, Terrer et al. (2021) suggested an incapability of forest soils to acquire more SOC under elevated CO_2 and increased biomass production, though observations were restricted to the top 30 cm. Based on our data collected over entire soil profiles, we propose that even though forest subsoils do have large capacities for further C sequestration as MAOC, their actual C storage will not fill these capacities due to multiple mechanistic constraints and thus limit their value as future C sinks.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the BonaRes repository at: https://doi.org/10.20387/bonares-rmtx-ttaj.

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