Links among warming, carbon and microbial dynamics mediated by soil mineral weathering

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Quantifying soil carbon dynamics is of utmost relevance in the context of global change because soils play an important role in land-atmosphere gas exchange. Our current understanding of both present and future carbon dynamics is limited because we fail to accurately represent soil processes across temporal and spatial scales, partly because of the paucity of data on the relative importance and hierarchical relationships between microbial, geochemical and climatic controls. Here, using observations from a 3,000-kyr-old soil chronosequence preserved in alluvial terrace deposits of the Merced River, California, we show how soil carbon dynamics are driven by the relationship between short-term biotic responses and long-term mineral weathering. We link temperature sensitivity of heterotrophic respiration to biogeochemical soil properties through their relationship with microbial activity and community composition. We found that soil mineralogy, and in particular changes in mineral reactivity and resulting nutrient availability, impacts the response of heterotrophic soil respiration to warming by altering carbon inputs, carbon stabilization, microbial community composition and extracellular enzyme activity. We demonstrate that biogeochemical alteration of the soil matrix (and not short-term warming) controls the composition of microbial communities and strategies to metabolize nutrients. More specifically, weathering first increases and then reduces nutrient availability and retention, as well as the potential of soils to stabilize carbon.

The potential of soils to sequester carbon (C) can vary greatly depending on soil development and how weathering interacts with biological processes that (re)cycle essential nutrients and elements12–14. Because of this complexity, assessing the development of soil C stocks and land-atmosphere exchanges15–17 remains one of the least constrained components of the terrestrial C cycle18–21. This is partially due to differing timescales of the processes involved. On the one hand, gross short-term fluxes of C between soil and atmosphere are dominated by processes involving non-protected C, such as respiration of plant residues, root exudation and rhizodeposition. As a result, many studies focus on short-term greenhouse gas fluxes. Studies on the effects of warming on plants and soils hardly exceed time series longer than years to decades22–24. Even shorter (years at maximum) are studies that focus on the interactions of microorganisms with the soil mineral matrix. On the other hand, the majority of C stored in soils persists and cycles within soils for decades to millennia, mainly through interactions with secondary soil minerals25 that are driven by mineral weathering operating on longer timescales14. While the importance of the mineral matrix for C stabilization is recognized1, the links between short-term processes determining the C exchange between land and atmosphere and long-term processes involved in soil genesis are not well studied.

On a chronosequence of felsic, granitic river terrace landforms covered by natural grassland and ranging in age since deposition from 0.1 to 3,000 kyr, we studied the co-evolution of biotic, mineralogical, geochemical and physical processes in the main rooting zone (0–15 cm depth) of soils. This chronosequence constitutes a space-for-time substitution to observe variability in soil development, across trajectories of soil mineral transformation (from primary to secondary minerals) that were developed under similar conditions (that is, semi-arid climate, grassland vegetation, parent material of similar origin), but experienced different duration and intensity of mineral weathering. By combining temperature sensitivity of the specific potential heterotrophic soil respiration (SPR, potential maximum of heterotrophic soil respiration at a specific temperature, normalized to the C content of the respective bulk soil and measured at 60% water holding capacity) with biogeochemical soil properties, soil microbial activity and community composition, we constrained the magnitude of change in soil C turnover, stocks and temperature sensitivity resulting from mineral transformation along this chronosequence (more information on the experimental approach can be found in Supplementary Section 1.1 and Supplementary Fig. 2).

Weathering effects on C cycling and microbial communities

Along the 3,000 kyr chronosequence, averageground net primary productivity (ANPP) and root biomass C decreased by about 35% and more than 50%, respectively, with terrace age (Table 1). However, this decrease is nonlinear: ANPP and root biomass C begin to decrease on older terraces only after reaching a
maximum peak in root C productivity at the 3 kyr and 35 kyr terraces, where soil fertility, indicated by potential cation exchange capacity (CEC\textsubscript{\text{CEX}}), and mineral specific surface area (SSA) are highest. To explain this nonlinearity, we must understand the dynamic shift from a nutrient-abundant to a nutrient-limited system. Primary minerals provide key nutrients to biota; whereas secondary minerals can not only stabilize organic molecules and nutrients for later release, but also enable soil aggregation that occludes nutrients from plant or microbial availability\cite{12,13}. On young terraces (0.1–3 kyr), nutrients are readily released from primary minerals through weathering. At intermediate weathering stages (35 kyr), primary minerals continue to weather, thereby depleting the soil nutrient reserve and decreasing the importance of primary minerals as a source of soil fertility. However, weathering produces highly reactive, clay-sized secondary minerals such as expandable smectite and vermiculite clays that are capable of stabilizing nutrients\cite{14} due to a large increase of SSA compared with their parent minerals, hence reducing nutrient losses through leaching. Both stages provide suitable conditions for plant growth and high C input, where nutrients are still abundant and readily available. As weathering continues to alter secondary minerals, it creates less reactive minerals such as non-expandable kaolinite and halloysite clays, less capable of releasing and/or stabilizing nutrients in soils. Importantly, we also observed a strong decrease of aggregation with terrace age, thereby suggesting a decrease in potential soil C stabilization with soil age (Fig. 1a,b and Table 1). Carbon density in the main rooting zone (0–15 cm soil depth) decreased fourfold, peaking at the 3 kyr terrace (6.6 ± 0.6 kg C m\textsuperscript{-2}) and being lowest at the 3000 kyr terrace (1.6 ± 0.5 kg C m\textsuperscript{-2}). A large part of this decrease in C density was related to decreases in unprotected, plant-derived C (that is, coarse particulate organic matter) and G in aggregates (physically protected C), while C associated with finer soil particles (C predominantly protected by organo–mineral interaction) was less affected (Fig. 1a; a more detailed discussion on weathering and stabilization mechanisms can be found in Supplementary Section 1.2). Thus, on older terraces (300–3000 kyr), a soil environment similar to what is commonly observed in deeply weathered systems all over the world was created\cite{15}, leading to sharp decreases in soil fertility as indicated by decreasing CEC and C stabilization potential as indicated by lower SSA and aggregation potential (Table 1 and Fig. 1). As low soil fertility limits plant growth and thereby C input, C stocks are not only lower due to less C stabilization but also due to less C input (see Supplementary Sections 1.3 & 1.4 for details and discussion on mechanisms of geochemical soil alterations; Supplementary Fig. 4).

Notably, the shift from a nutrient-abundant to nutrient-limited system is not only reflected in the trajectory of ANPP and root biomass C, but also in adaptations of microbial communities\cite{16}. For example, we observed a significant increase of amino sugar ratios (glucosamine/galactosamine (GlcN/GalN)) at the oldest 3000 kyr terrace, reflecting a restructuring of microbial communities from bacteria-dominated to fungi-dominated\cite{17} communities, even though the exact direction of this shift is debated\cite{18} (Fig. 2a). Numerous studies indicate lower C source availability and quality stimulate the development of a microbial community that is dominated with niche specialists\cite{19}. Hence, it is reasonable that we found a fungi-dominated community at the oldest terrace that is equipped with strategies to produce extracellular enzymes to degrade more complex organic matter and able to function in more acidic environments\cite{20} (see Supplementary Section 1.5 for more discussion on the interpretation of amino sugars). Furthermore, microbial acquisition strategies through extracellular enzymes along the chronosequence shift from C towards N and P mining with age (Fig. 2b). We argue that the decreases in C:N and C:P acquisition ratios reflect primarily a change in substrate quality as

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|}
\hline
Terrace & ANPP (g C m\textsuperscript{-2} yr\textsuperscript{-1}) & Root biomass (g C m\textsuperscript{-2}) & SSA (m\textsuperscript{2} g\textsuperscript{-1}) & C coverage of MS (%) & Total C (kg m\textsuperscript{-2}) & Total N (kg m\textsuperscript{-2}) & Total P (kg m\textsuperscript{-2}) & Bulk density (g cm\textsuperscript{-3}) & Gravel (>2 mm) (%) \\
\hline
0.1 & 100.2 ± 0.8 & 205.5 & 81 ± 0.02 & 65 & 4.6 ± 0.4 & 0.4 ± 0.4 & 0.22 & 15.2 & 6.8 & 1.3 ± 0.01 & 5.8 ± 2.9 & \\
3 & 85.6 ± 12.7 & 186.0 & 30.3 ± 0.19 & 58 & 6.6 ± 0.6 & 0.6 ± 0.1 & 0.14 & 13.4 & 6.6 & 1.21 ± 0.04 & 7.6 ± 4.1 & \\
35 & 86.3 ± 10.0 & 268.5 & 26.7 ± 0.07 & 26 & 4.7 ± 1.8 & 0.4 ± 0.01 & 0.11 & 20.8 & 5.9 & 1.28 ± 0.02 & 7.6 ± 2.8 & \\
300 & 80.1 ± 5.6 & 165.0 & 4.3 ± 0.11 & 46 & 3.2 ± 0.17 & 0.3 ± 0.02 & 0.07 & 4.6 & 6.3 & 1.65 ± 0.14 & 7.9 ± 7.8 & \\
3,000 & 65.7 ± 0.1 & 103.5 & 9.0 ± 0.12 & 36 & 1.6 ± 0.5 & 0.2 ± 0.07 & 0.09 & 4.5 & 4.7 & 1.62 ± 0.06 & 4.26 ± 3.9 & \\
\hline
\end{tabular}
\caption{Overview of soil fertility and biomass production dynamics as a function of soil age through weathering.}
\end{table}

Sampled soil depth: 0–15 cm; see Supplementary Information for subsoil information; ANPP: SSA, bulk density and gravel are mean ± s.d. Total C, N and P are average total (organic + mineral) carbon (C) (mean ± s.d.), nitrogen (N) (mean ± s.d.) and phosphorus (P); MS: mineral surfaces. For pH and CEC\textsubscript{\text{CEX}} deviations among duplicate measurements were consistently <5%.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig1.png}
\caption{C stocks and key mineralogical parameters in soils in response to weathering. a. Distribution of soil C density among fractions. Unprotected, coarse particulate organic matter; not associated with minerals; mAgg, microaggregate-associated C; s + c, non-aggregated silt and clay-associated C. Error bars represent standard deviation. b. FE\text{\textsubscript{m}}/F\text{\textsubscript{Dox}} ratios show total Al, Fe and Mn content as measure of weathering degree along the sequence including percentage of low-activity (end member) clays to total clay fraction. Deviations among replicate measurements of FE\text{\textsubscript{m}}/F\text{\textsubscript{Dox}} ratios <5%. All displayed soil parameters are for the 0–15 cm depth increment; for more information on weathering indexes and fractions, see the Supplementary Information and Methods.}
\end{figure}
P and N become increasingly limited. These C:P and C:N shifts are consistent with a decreasing importance of plant material as a nutrient source for heterotrophic microorganisms towards an increasing importance of recycled microbial residues in older soils; the latter is supported by C radioisotope data (Supplementary Table 4) and indicated by relative increases in N-acetylglucosaminidase activity (an enzyme that mines key components of microbial cell walls). The growing importance of nutrient recycling in older soils is further supported by decreasing fresh plant C inputs with terrace age (Table 1) leading to a general decrease of percent modern carbon (pM-14C) and slower C turnover in soils (Fig. 3b).

Interplay of weathering and warming for soil respiration

The SPR increased by ca. 37% for the bulk soil, 34% for the silt and clay-associated fractions and by more than 66% for the aggregated C with age along the sequence. While no significant difference in temperature sensitivity of SPR (expressed as Q10) was observed for the bulk soil (Q10 = 1.9 ± 0.2) along the chronosequence, Q10 of SPR for C associated with soil aggregates decreased by almost 60%, limiting the effect of warming-related C release on older terraces (Fig. 3a and Supplementary Table 6). Remarkably, no statistically significant difference in enzyme activity rates at 20°C compared with 30°C incubation temperature were observed, indicating that differences in acquisition strategies in soil are more strongly tied to changing soil properties due to weathering (and may include long-term adaptation to climate) than to short-term temperature conditions (Fig. 2b). Noteworthily, the absolute activity of enzymes at 20°C compared with 30°C averaged over all measured enzymes (see Methods) was also not significantly different (data not shown). Comparing the radiocarbon content of bulk soil to that of respired CO2 revealed that respired CO2 of younger (0.1–3 kyr) terraces was not significantly different from that of the bulk soil, with no significant difference between the 20°C and 30°C incubation experiments. The radiocarbon content of CO2 respired from 35 kyr terrace soil, however, was significantly lower than that of bulk soil for the 20°C incubation (–1 to –7% pM-14C in CO2 versus soil). Older terraces (300–3,000 kyr) showed a different response: radiocarbon content of CO2 was generally higher compared with bulk soil (+12 to +7 pM-14C in CO2 versus soil), again with no significant difference between the 20°C and 30°C incubation experiments (Fig. 3b). This pattern indicates that terrace age affected the uptake and respiration of old C versus young C, but incubation temperature did not. With increasing terrace age, microbial respiration is discriminating against older C sources. A possible explanation for this discriminative respiration could be the fast incorporation and mineralization of new C into microbial biomass while older mineral-associated C remains largely untouched due to higher energetic barriers that cannot be overcome in old, nutrient-poor soils, with organic matter of predominately microbial origin stabilized with minerals. However, while there might be an effect of (bio)chemical structure and age of C molecules on the isotopic signature of respired C (Fig. 3b), it is not as an important mechanism to explain C stabilization or release in these soils compared with aggregation or mineral association of C (Fig. 3a).

From our findings along the chronosequence, we draw several overarching conclusions (Fig. 4a). The progression of mineral weathering affects all compartments of the soil C cycle, with control over C inputs and loss, as well as persistence and temperature.
sensitivity of soil C. Carbon stocks in soils decrease due to lower C inputs as a result of changes in soil fertility but also due to reduced capacity of soil minerals to stabilize and retain C. While microbial physiology directly controls the response of soil C storage and turnover to warming, microbial communities and their acquisition strategies for C, N or P are governed by availability and accessibility of these elements, which is controlled by soil weathering. Thus, weathering diversifies the microbial response to warming (Fig. 3 and Supplementary Table S3) and decomposer communities as a whole adapt well to this wide range of geochemical soil conditions. Furthermore, the rates at which extracellular enzymes are contributing to nutrient mining were affected by the age of the terrace sediment and the soils developed on them, but not by incubation temperature. Hence, in our study of a wide range of geochemically different soils under similar land use, vegetation and climate, C storage was predominantly controlled by long term weathering processes. From a conceptual point of view, the limitation and potential of soils to sequester C is input driven in soils with limited stabilization capacities (very young and very old soils due to the lack of reactive mineral surfaces, unlike soils of intermediate weathering stages). However, young soils are capable of releasing nutrients in great abundance from primary minerals leading to high plant biomass yields while old soils lack this source of nutrient input (Table 1 and Supplementary Table S2). Decomposer communities simultaneously adjust to a wide range of soil and environmental conditions. Importantly, our results show that shorter-term responses of the C cycle, such as changes in respiration or biomass production, are not predictable from initial soil C stock assessments plus warming alone. Older soils are less responsive to warming than younger soils as the latter have generally less reactive, highly weathered minerals (kaolin group clays, chlorites and so on) and the age of soil C is not necessarily a good indicator for potential stability, hence old C in soils is not more recalcitrant per se13-15 (more discussion on the weathering-related interplay of capacity and demand for C stabilization along the sequence can be found in Supplementary Section 1.5). Looking beyond the chronosequence The geochemistry along this sequence reflects conditions that can be found in major, global geo-climatic ecoregions and have the potential to be greatly altered by climate change and human activity (Fig. 4b,c). For mineral soils, the largest potential responses to warming can be expected in the youngest soils that are the least exposed to weathering, as, for example, in higher latitudes. If climatic limitations to chemical weathering would disappear, these areas will see a great change in C cycling within years to decades41. Similarly, land conversion and erosion can lead to the removal of weathered material, changing the mineral matrix in which biological processes take place and drastically change the geochemical conditions for C stabilization. Hence, mineral transformation due to weathering impacts the functional link between microbial community structure and soil geochemistry and mediates the soil response to warming as well as C and nutrient dynamics in soils over large spatial and long temporal scales41.

Methods
Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41561-018-0168-7.
References


Acknowledgements

This research was financed in the framework of the BELSPO funded research action P2 as part of project P2/14 SOGLO. Further financial support has been given by: UC Merced, UC-Irvine, ETH Zurich and Aarhus University. Special thanks to H. Maidlau (Marine Scientific Editing) for language proof reading, to M. Schülz (US Geological Survey) for providing a USGS internal review of this manuscript, as well as the Soil and Water Conservation Society and the US Department of Agriculture for imagery of microbial communities used in Supplementary Fig. 2.

Author contributions

S.D. and A.A.B. designed the research and co-authors are listed in alphabetical order. S.D., A.A.B. and J.W.H. conducted sampling campaigns. S.D., C.A., L.F., J.Schneider, I.Six, C.V. and E.N. and M.G. collected, analysed and interpreted the data. All authors contributed to the writing of the paper.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41461-018-01847-7.

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Methods

Study site selection and sampling. Soil samples were taken in December 2013 from rangeland under extensive land use along a sequence of alluvial terrace deposits of the Merced River in the Calaveras Big Trees Valley (Merced County, CA, USA) (Supplementary Fig. 1 and Supplementary Table 1). Our sites are located on the terraces east of the Merced River across an area of about 25×25 km². Soil profiles descriptions were done using the Food and Agriculture Organization (FAO) guidelines and classified using the World Reference Base for Soil Resources (WRB)16. Partly, this campaign resampled terraces and sites where legacy data on soil mineralogy and geochemistry was available17–20. Where new sites have been selected, ratios of Ti/Zr and Fe₂O₃/FeO₀.₉₂ have been measured as indicators for the weathering stage of the deposits and compared with the existing mineralogical data to constrain the placement of the selected sites along the timeline of the terrace sequence. The deposits consist of felsic, magmatic and metamorphic material of similar geochemistry and of fine sandy and silty texture, mobilized in the Sierra Nevada mountain range21. While no consistent changes are detectable among the fine soil textural, an increase of coarse fragments (quartz pebbles) has been observed in the oldest terrace (Supplementary Table 1). The terrace sequence is situated under the typical semiarid climate of the Californian Central Valley with long term climate data indicating a relatively climate similar (that is, the Köppen climate class, but fluctuations in mean annual temperature (MAT) and mean annual precipitation (MAP)) for the time period covered by the terrace sequence and very strong climatic similarity over the course of the Holocene with no major fluctuations in MAT and MAP10–14, 22–24 (Köppen, BSk, MAP 18.3 °C, MAP 315 mm). While the terraces are mostly flat, plateau-like structures, the incision of the Merced River has created noteworthy alitudinal differences from the oldest (≈220 m above sea level) to the youngest (≈50 m above sea level) terrace, with the youngest terrace being geochemically equivalent to the stream deposit and riverine gravel. Thus, none of the investigated terraces is influenced by ground water or stream water dynamics. The lowest terrace is situated in a dry valley, created by an accidental flash flood resulting from the breach of a nearby water reservoir in 1914. For five different terraces, covering the complete temporal range of the terrace sequence (0.1–3,000 ky), 1 m² sized soil profiles were excavated covering dystric Fluvisols (younger terrace) and chromic Alisols (other terraces). At each terrace, from three replicate soil profiles, soil samples were taken from the main rooting zone of soil (topsoil ≈0–15 cm). After sampling, soils were dried at 40 °C, homogenized and sieved at 2 mm with roots >3 mm removed. Data on biogeochemical soil variables are available for C-deprived subsol (>15 cm) in the Supplementary Information for context and further discussion. Where not described differently, all follow-up laboratory experiments and extractions are reported as the mean of three replicates.

Aboveground net primary productivity (ANPP) and root C biomass. The dominant vegetation for most of the period of soil formation at the study sites are remnants of the Californian Central Valley perennial bunchgrass ecosystems with no major differences in species composition and dominance. Today’s vegetation is now mainly European annual grasses. ANPP for the study region has been estimated based on normalized difference vegetation index (NDVI) data for 2007–2015 according to ref. 15. Briefly, a five-year average of the integral of the NDVI (NDVI integral) was used to derive spatially aggregated values of ANPP. The NDVI averaged for 2010–2015 was derived from the MOD13Q1 Modis (TERRA) vegetation indices 16 global 250 m product accessed from NASA Land Processes Distributed Active Archive Center at the USGS EROS Resources Observation and Science Center through the Google Earth Engine platform17. Carbon biomass of ANPP has been estimated using average reference values from literature for grassland in the study region (45±3 ± 0.2% C)20. Root biomass C ([Cg (mm)]) was derived from root abundance calculations21 using dry sieving to isolate >2 mm roots assessed at the time of sampling. Briefly, for each site and soil horizon, five roots were categorized according to size and abundance and then translated into mg C based on an average of 48% C of the total fine root biomass.

Physical soil parameters and visual observations at profile pits. Soil texture was analysed using a Mastersizer 2000 laser diffraction particle size analyser (Malvern Instruments) and correction factors for the underestimation of clay in silty soils were applied18. Bulk density was analysed according to ref. 19. Redoximorphic features have been identified through visually qualifying the relative amount of visible amounts of FeMn nodules, concretions and Fe reduced areas in soil horizons <1 m during the sampling campaign (Supplementary Table 2).

Geochemical soil parameters. Elemental composition and degree of weathering. Total reserve (the sum of all bioavailable elements in Ca, Na, K, Mg, Mn, and Fe) was measured following ref. 23. Total reserve in base cations can be used to compare soil material of similar depth along the terrace sequence and to evaluate the weathering degree of soil material by assessing the relative loss of Ca, Na, K, Mg, Mn and Fe during weathering. The total reserve was determined by inductively coupled plasma–atomic emission spectrometry (ICP-AES) on the bulk soil after borate fusion23. The same procedure was used to determine the total concentrations of several other elements such as P, Mn, Ti and Zr. An extraction of total pedogenic organo–mineral associations and oxy-hydroxides was carried out in duplicate using Dithionite–citrate–bicarbonate (DCB) at pH 8. This allows assessment of the amount of Fe and Al-bearing pedogenic phases and Fe and Al through pedogenesis. DCB extractable components were analysed for Al and Fe content by ICP-AES. In addition, the degree of soil weathering was estimated by comparing the ratios of Ti/Zr and Fe₂O₃/FeO₀.₉₂ with Ti and FeO₀.₉₂ enriching over time relative to the Zr and Fe₂O₃ content, respectively24.

pH, CEC and base saturation. Soil pH values were determined as the mean of two measurements per sample after a response time of 30 min and 24 h, respectively, in a 1:2.5 soil (weight)/solution (volume) ratio in 25 mL of 0.01 M CaCl₂ potentiometrically with a glass electrode using a portable multiparameter Meter HI98308 (Hanna Instruments). CEC₅₀ was determined by quantifying N, Iₑ, exchanged with 2 M KCl after saturating cation exchange sites with ammonium acetate25 buffered at pH 7.0. Exchangeable Al was extracted by 1 M KCl solution and determined colorimetrically. The total percent base saturation, defined as the relative availability of all base cation (that is, the sum of all base cations Na, K, Mg, Ca) for CEC₅₀, was calculated in percent of CEC₅₀.

Clay mineralogy. The mineralogy of the clay-sized fraction was determined by X-ray diffraction (Cu Kα, Dr. Brucker Advance) after K⁺ and Mg²⁺ saturation, ethylene glycol solution and thermal treatments at 300 and 550 °C (ref. 19). Clay minerals were classified and peak identified according to ref. 26. Quantitative analyses were performed using the Rietveld method through the software package Siroquant V4.0. Refinement of Rietveld parameters was carried out following the instructions provided in the ‘Siroquant V4.0 Technical and Clay Manuals’ until a chi-square value < 3 was obtained. Key primary minerals that can interfere with the spectra (quartz) and act as sources for clay formation such as amphiboles (brookite/mica), feldspars (cummingtonite, biotite) and biotite, etc. were identified and used to derive the non-expandable 2:1 layered illite and chlorite and the non-expandable 1:1 layered kaolinite. Note that no distinction has been made between primary and secondary chlorite. In a final step, secondary clay mineral abundance was compared along the terrace sequence.

Specific surface area (SSA). The SSA of the bulk soil was determined by N₂ adsorption (Autoorb-1 analyser, Quantachrome) using the Brunauer-Emmett-Teller (BET) equation27, which is a standard method for measuring SSA of soils based on physisorption of N₂ gas on a sample at 77 K. Sample size (<1 mm, removing any visible root and coarse particulate organic matter fragments) were measured before and after C removal to assess the degree of C coverage of mineral surfaces (Supplementary Table 2 and Supplementary Section 1. C) was removed by oxidation with a 10% H₂O₂ solution at room temperature adding H₂O₂ until no reaction was visible any more for 24 h. N₂ adsorption was measured in the relative partial pressure range (p/p₀) of 0.05 to 0.3. Samples were outgassed for 14 h under constant helium flow at 40 °C before measurements were carried out. Duplicate analyses were carried out to verify the repeatability of the experiment identified <5% relative uncertainty among all investigated samples.

Soil carbon fractionation and C dynamics. All samples have shown no reaction when treated with 10% TCA and are considered free of carbonates. Total soil C content is therefore interpreted as soil organic carbon only. A subsample of 80 g was fractionated in triplicate for each soil depth and layer to derive functional C fractions. We used a method based on the conceptual C fraction model proposed by ref. 28 (Supplementary Fig. 3). Briefly, the scheme consists of a series of physical fractionations techniques applied to isolate C fractions, differentiated by stabilization mechanisms (chemical, biochemical and physical), which can also be associated with different turnover times and C stability. Using a microaggregate isolator, C was fractionated into coarse particulate organic matter C (≥250 μm), microaggregate-associated C (≥25–53 μm) and non-aggregated silt and clay C (<53 μm) (for more detail see Supplementary Table 3 and Supplementary Section 1.7). To better understand the C composition of the different isolated mineral associated fractions, we analysed the C concentration of a fraction as well as its stable isotopic composition (δ¹³C and δ¹⁵N) and ratio and compare it with the respective bulk soil measurements using the sequential approach. Total soil root and litter was measured in 1 g ground subsamples using a dry combustion analyser (VarioMax CN, Elementar) with a measuring range of 0.2–400 mg g⁻¹ soil (absolute C or N mass in sample) and a reproducibility of <0.5% (relative deviation). Recovery rates exceeding 97% and 91% were obtained for the soil mass as well as C and N concentrations, respectively, across all soils.

Temperature-sensitive soil incubation. Three replicate samples of 15 g of bulk soil, as well as isolated mAgg and silt and clay fractions from each study site were incubated at two different temperatures (20°C) and two oxygenation states (10% O₂ pre-incubation (including 10 d pre-incubation at 20 °C for both sets) while keeping temperature and moisture (60% soil water holding capacity) constant for the length of the experiment, by adding demineralized water to the samples throughout the length.
of the experiment when necessary) (for more detail see Supplementary Table 5 and Supplementary Section 1.8). Incubation data were used to derive the SFR \(^{14}C\), expressed as \(^{14}C\)-per unit soil C, at the respective temperature level of the incubation. \(^{14}CO_2\) production was analysed as the average SFR over the complete period of the incubation (pre-incubation excluded) measured at day 7, 30 and 60. Furthermore, SFR data were used to calculate the \(^{14}C\), of the heterotrophic soil respiration (that is, the difference in SFR of the same aliquot samples at 20\(^\circ\) to 30\(^\circ\)C incubation temperature). Note that for incubating isolated fractions, 3g of sample was mixed with 12g of C-free silty/sandy sand soil material, representing the mean texture of the bulk soil (see Supplementary Table 1). Each sample was put in a 355 mm \(\times\) 1 mm sealed mason jar with no further additives. To avoid \(^{14}CO_2\) losses, each mason jar was sealed with Silastic O-rings. After sealing, the mason jars were flushed with fresh air after each measurement. Samples of the gas mixture within the incubation jars were taken periodically every 3 to 7 through the experiment and analysed for \(^{14}CO_2\) concentration using a gas chromatograph (Shimadzu GC-14B, Shimadzu Scientific Instruments).

Isotopic composition and percent modern \(^{14}C\). Using \(^{14}C\) radiocarbon dating, we estimate C turnover of the organic material associated with bulk soil, mAgg and non-aggregated sil and clay fractions along the sequence to study relative differences between soils and fractions. In addition, at the last sampling point for \(^{14}CO_2\) at the end of the incubation experiment, gas samples were analysed for \(^{14}C\) (expressed as the percentage of modern C (PM, \(^{14}C\)-PM)) to identify differences in the \(^{14}C\) signature of mineralized C compared with the incubated soil and soil C fractions. We used these relative differences to evaluate (1) the effectiveness of different fractions in stabilizing C against decomposition, (2) to estimate the input of fresh C through litter decomposition and (3) to estimate the residence of C against mineralization under different temperature regimes. In short, roughly 1.25 mg of \(^{14}CO_2\) was isolated per mason jar at the end of the incubation period. The sample preparation backgrounds have been subtracted, based on measurements of \(^{14}C\)-free coal. The radiocarbon signature of the graphite was measured with accelerator mass spectrometry (NCS.05MM 1.35DAMS-2 AMS system) at the Keck- Carbon Cycle AMS facility at University of California, Irvine (California, USA). Results have been corrected for isotopic fractionation according to the conventions of ref \(^{14}C\), with \(^{8}O\) values measured on the prepared graphite using the AMS spectrometer. Radiocarbon concentrations are given as fractions of a modern standard annual age and are normalized to the C content of the respective samples. Parallel analyses were conducted using a liquid chromatography pump (Surveyor MS-Pump Plus, Thermo Scientific) mounted with a PA30 CarboPac analytical anion-exchange column (3 x 150mm, 6.5um) and a PA20 guard column (Thermo Scientific), detection was done using IRMS coupled to the liquid chromatography device through an interface (LC.ISOLINK Thermo Scientific). In soils, only Gln, Glnh, mannosamine and muramic acid are found in quantifiable concentrations. Differences in the Gln/Glnh ratios were interpreted as qualitative changes in microbial community structure with higher ratios indicating a relative shift towards fungal residues and lower ratios a relative shift towards bacterial residues, respectively (for more detail see Supplementary Table 3 and Supplementary Section 1.4). Each sample was extracted in duplicate. Due to the very low contents in muramic acid in the extracts, quantifiable analyses of amino sugars were done for Gln and Glnh only. An analysis of the repeatability between replicated extracts of selected samples shows that the limit for quantifying the amino sugars reliably was 100 and 250 \(\mu\)g amino sugar per g soil for Gln and Glnh, respectively. Hence, we limit our analysis of amino sugar data to observations that exceed the letter mentioned amino sugar content and to a description of trends within the dataset.

Potential extracellular enzyme activity rates. Potential enzyme activity assays were determined fluorimetrically and photometrically using modified microplate assays \(^{14}C\) in soils before (10 d acclimatization at 20\(^\circ\)C, 50% water holding capacity) and after incubations for 60 d at 20\(^\circ\)C and 30\(^\circ\)C, respectively. The assays were conducted with soil aliquots (1.0 g) dispersed in 100 ml of sodium acetate buffer (100mM, \(pH\) 5.5) and incubated for 1 min. For the fluorimetric enzyme assay, 200 ml of soil suspension were pipetted into black microplates in 5 replicates. Each well received 50 \(\mu\)l of MUF (4-methylumbelliferyl)-beta-glucuronide substrate; MUF-beta-glucuronido. For the photometric enzyme assay, MUF-beta-glucuronido, for beta-glucosidase (BG, hydrolytic C mining enzyme) and MUF-N-acetyl-beta-D-glucosaminido for N-acetylglucosaminido (NAG, hydrolytic N mining enzyme). t-leucine/7-amido-4-methylcoumarin was used as substrate for leucine-amino-peptidase (LAP, hydrolytic N mining enzyme). MUF-phosphate was used for phosphatase activity (PIIIOS, hydrolytic P mining enzyme). Microplates were then incubated at 20\(^\circ\)C and 30\(^\circ\)C, respectively, for 140 min. Fluorescence was measured with excitation at 365 nm and emission at 450 nm, activity was quantified against standards of MUF and 7-amido-4-methylcoumarin. Phenoloxidase (POX, oxidative enzyme, mining various nutrients) and peroxidase (PEN, oxidative enzyme, mining various nutrients) activities were determined photometrically using 3,3-dibromotyrosine/t-l-alanine (DOPA) as substrate. For this assay, triplicates of 1 ml of the soil suspension received 1 ml of a 20mM substrate solution. Activity was calculated against the respective 250 \(\mu\)l into duplicate microplates for POX and PEX determination. To determine PEN activities, one set of plates additionally received 10 \(\mu\)l of 0.3% H\(_2O\)\_2. Then activity was measured photometrically at an absorbance of 450 nm at the start and after 10\(\_\)5 of incubation at 20\(^\circ\)C and 30\(^\circ\)C, respectively. POX activity rates were calculated as the difference in absorption over incubation time in the plates without H\(_2O\)\_2. PEN activity rates were calculated as the difference between the changes over time of POX activities and the activities of the plates with H\(_2O\)\_2 additions.

We tested whether terrestrial age or incubation temperature affects microbial functioning using enzyme activity rates per unit soil C before and after incubation at 20\(^\circ\)C and 30\(^\circ\)C. Moreover, we tested whether microbes adjust their nutrient acquisition strategy to different temperatures by calculating nutrient acquisition rates based on potential enzyme activity rates. The C:N acquisition by enzymes was calculated as the sum of log-transformed BG and CBH activities over the sum of log-transformed activities of LAP and NAG at the end of the incubation experiment. Similarly, C:P acquisition was calculated as the sum of log-transformed APase and CHBP activities divided by the log-transformed PIIOS activity. The relative difference along the chronosequence and among the ratio is of great interest for interpreting microbial nutrient acquisition strategies.\(^{14}C\)

Implementation and statistical analyses. All statistical analysis was realized using SPSS 24.0\(^{14}C\) and R 3.3.2.\(^{14}C\). Differences between the means of classes have been performed using multi-group analysis of variance (ANOVA), Bonferroni corrections, Tukey's honest significant difference test and Tamhane\(_T2\). Linear regressions were done to test correlations between the abundance of the reactive soil phases and C in the bulk soil and for the isolated fractions. In addition, stepwise linear regressions were used to estimate the best prediction model for Q\(_{\text{sp}}\) and SFR at 20\(^\circ\)C and 30\(^\circ\)C for the bulk soil and fractions using the abiotic, mineralogy-related parameters and C input variables assessed in this study (for more details on these results, see Supplementary Table 6 and Supplementary Section 1.8). These models were tested for the (1) homogeneity of model residues and (2) normality of variables, restricted to (3) models were the variable inflation factor for each variable is below 10. Where (4) model validation improved the overall model performance (corrected R\(^2\)) by >0.1. All statistical tests used in this study were evaluated using \(P < 0.05\) as the level of significance. Please note that throughout the manuscript C mass refers to the C concentration of a sample multiplied by the mass of the fraction it is extracted from or multiplied by the bulk density in case of C mass of the bulk soil. Measured values were normalized to the C-content of the respective soil or fraction (that is, amino sugar-soil C\(_{\text{soil}}\)-C\(_{\text{soil}}\)-C\(_{\text{soil}}\) and so on) to evaluate the relative differences in the relationship of certain parameters to total C in soil. To test whether soil age, soil depth or incubation temperature affect microbial functioning, we conducted a permutation ANOVA ("adonis" using the包 package ‘vegan’) based on Euclidean distance matrices using enzyme activity rates per unit soil C. Results are displayed in a non-metric multidimensional scaling plot.

Data availability. The soil carbon-related datasets generated and analysed during this study are available from the Powell Center soil carbon database and are available from the corresponding author upon request. All other data are included in this published article and its Supplementary Information files and no third-party data have been used.

References


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