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The impact of Last Glacial climate variability in west-European loess revealed by radiocarbon dating of fossil earthworm granules

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The characterization of Last Glacial millennial-timescale warming phases, known as interstadials or Dansgaard-Oeschger events, requires precise chronologies for the study of paleoclimate records. On the European continent, such chronologies are only available for several Last Glacial pollen and rare speleothem archives principally located in the Mediterranean domain. Farther north, in continental lowlands, numerous high-resolution records of loess and paleosols sequences show a consistent environmental response to stadial-interstadial cycles. However, the limited precision and accuracy of luminescence dating methods commonly used in loess deposits preclude exact correlations of paleosol horizons with Greenland interstadials. To overcome this problem, a radiocarbon dating protocol has been developed to date earthworm calcite granules from the reference loess sequence of Nussloch (Germany). Its application yields a consistent radiocarbon chronology of all soil horizons formed between 47 and 20 ka and unambiguously shows the correlation of every Greenland interstadial identified in isotope records with specific soil horizons. Furthermore, eight additional minor soil horizons dated between 27.5 and 21 ka only correlate with minor decreases in Greenland dust records. This dating strategy reveals the high sensitivity of loess paleoenvironments to Northern Hemisphere climate changes. A connection between loess sedimentation rate, Fennoscandian ice sheet dynamics, and sea level changes is proposed. The chronological improvements enabled by the radiocarbon "earthworm clock" thus strongly enhance our understanding of loess records to a better perception of the impact of Last Glacial climate changes on European paleoenvironments.

millennial-timescale climate change | radiocarbon dating | earthworm calcite granules | Last Glacial loess | Europe

Millennial-timescale alternations of Greenland stadials (GSs) and Greenland interstadials (GIs) forming Dansgaard-Oeschger cycles are global climate changes well-expressed in Last Glacial ice cores (1) and marine sediment proxy records (2), but their causes and mechanisms are still debated (3–5). Modeling results show that the European climate was particularly impacted by switches between weakened or enhanced modes of the Atlantic Meridional Overturning Circulation through the transportation of North Atlantic air masses by Westerlies (6). Still, there are growing evidences that Greenland high-resolution climate records do not exhibit the full low-latitude climate variability, especially during cold periods (i.e., no distinction of Heinrich events) (7, 8). There is thus an urgent need for well-dated high-resolution records of millennial-timescale climatic variability at midlatitudes. Although many speleothems, marine, and lake sediment records are available in the Mediterranean domain (9–11), continental regions of Northern Europe are not

adequately documented, and very few radiometric ages are available for records north of 45° N. However, aeolian periglacial deposits forming the European Loess Belt (Fig. 1) are the most widespread sedimentary archive available for the detailed study of Last Glacial climatic and environmental changes in continental Europe (12).

The best loess sequences, owing to their high sedimentation rate that can reach 0.5–2 m/ka during the 35- to 17-ka time interval (13, 14), are indeed well-suited to study millennial-timescale environmental changes. High-resolution stratigraphy, paleopedology, grain size, magnetic properties, malacology, and organic and isotopic geochemistry can be used to reconstruct rapid variations of aeolian dynamics, relative temperatures, paleoprecipitation, and vegetation cover during the Last Glacial (15). During the Middle and Upper Pleniglacial [i.e., roughly marine isotope stage 3 (MIS 3) and MIS 2], the cyclical alternation of loess units with 0.3- to 0.5-m-thick tundra gley horizons (i.e., gelic gleysols resulting from the seasonal thaw of a permafrost active layer in tundra environments) or arctic to boreal brown soils (i.e., cambisols characterized by a slight weathering of the parent material in shrub-tundra environments) suggests a close connection with the succession of GS and GI identified in other archives. This link is supported by the strong relationship

Significance

Last Glacial millennial-timescale warming phases well-recorded in Greenland ice cores are relevant across the Northern Hemisphere. However, dating limitations in loess deposits inhibited characterizing their impact on the European Great Plain. Here, the radiocarbon dating of a large set of earthworm calcite granule samples from the Nussloch reference loess sequence (Rhine Valley, Germany) led to a straightforward chronological distinction of all soil horizons. Resulting correlations with Greenland interstadials between 50 and 20 ka also revealed more complex climate dynamics than interpreted from Greenland $\delta^{18}\text{O}$ records. This study is a fundamental contribution to understanding links between mid- and high-latitude climate changes and their spatial and temporal impact on paleoenvironments and prehistoric population settlement in Europe.

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the above two results discarded, all remaining ages define a chronological series with no statistically significant age inversion. Age reproducibility checks performed in tundra gley units Gm3 and G1a on P8 are positive (Fig. 3 and Table S1). Ages from arctic to boreal brown soils fit well among tundra gley ages. Ages from tundra gleys Gm3 and Gm1, located only 40 cm below the base of the Lohne Soil and the Upper Gräselberg Soil, respectively, do not seem rejuvenated as one might expect if bioturbation related to these more developed soils was important (Table S1). In absence of deep earthworm activity in these permafrost-free paleosols, dated granules might have mainly been produced by epigeic earthworms living close to the soil surface rather than anecic earthworms that usually dig deep burrows (25). Reliable ages are thus obtained from all tundra gleys and arctic to boreal brown soils as long as they are not affected by deep bioturbations from the topsoil. The regular distribution of radiocarbon ages throughout the loess profile indicates an almost continuous sedimentation between 50 and 20 ka. Sedimentation rates derived for the 45- to 21-ka interval are higher in loess units than in pedogenetic horizons. They increase from 0.19 mm/y for the Middle Pleniglacial to 0.33 and 1.12 mm/y for the early and full Upper Pleniglacial, respectively (Fig. 3), matching previous estimates based on luminescence ages and previous correlations with Greenland (14). We can now confidently establish correlations with other archives over the entire Nussloch sequence and especially after 30 ka.

Discussion

Two major changes in sedimentary and environmental dynamics are evidenced in the Nussloch loess sequence records. The first one around 30 ka presents a major limit marked by a sharp and strong increase in both sedimentation rate (Fig. 3) and grain size index (GSI) (14). These features observed in almost all of the loess sequences from Western to Eastern Europe (26–30) thus constitute a major marker level for stratigraphic correlations. This marker level at ~30 ka is, within limit uncertainties, synchronous with (i) a first step of expansion of the Last Glacial maximum (LGM) Fennoscandian ice sheet and mountain ice caps because of higher precipitations (31, 32), (ii) a significant drop in sea level from about –60 to –100 m (33), and (iii) a change from anastomosing to higher-energy braided channels in west European fluvial systems (34). This configuration induced a widening of deflation areas on the continental shelves of the North Sea and Channel and in large river valleys and a very strong increase in detrital particles available for Aeolian deflation and transport, enabling the deposition of markedly thicker loess units over Europe (35). A second marker level, observed around 23 ka at the top of tundra gley G7, is characterized by a sharp decrease in the GSI, an almost complete disappearance of the rich mollusk fauna of Nussloch (17), a shift from finely laminated to homogeneous loess in Western and Central Europe (26, 27, 30), and a significant increase in the sand content in Eastern Europe (28, 29). This marker level at ~23 ka, also recorded across the European Loess Belt, indicates a shift to markedly more arid conditions between 23 and 20 ka contemporaneous with maxima in ice sheet extension and volume. However, very low sea level and high-energy braided channels in the deflation areas persisted during this time interval, permitting high loess sedimentation rate (~1 mm/y) to be maintained in European loess profiles.

Furthermore, based on our radiocarbon chronology, reliable correlations between the pedostratigraphical sequence of Nussloch and Greenland climate proxy records over the 55- to 20-ka interval can now be established (Fig. 3). Each tundra gley and arctic to boreal brown soils correlates with a single GI within dating uncertainties (Methods). The only two exceptions are related to low sedimentation rates during the Middle Pleniglacial. The tundra gley Gm3, which is twice the thickness of Gm2 and Gm1, most likely stacks two successive tundra gleys formed during GI 11 and GI 10 (i.e., during the Hengelo interstadial) (36, 37). Similarly, the Lohne Soil appears likely as a stack of two soil horizons developed during GI 8 and GI 7c (i.e., during the initial phase of the Denekamp interstadial complex) (36). Our

chronology thus confirms and updates previous correlations (Table S2) based on Aeolian dynamic proxies. Around 50° N, Crag Cave speleothems (southwest Ireland) constitute the only other record of most Last Glacial interstadials. Indeed, their growth phases are induced by climate ameliorations contemporaneous to GI (38) as well as mollusk abundance increases in Nussloch soil horizons (17, 39). However, speleothem growth ceases during stadials, whereas loess deposition continues, hence revealing additional variability.

We can thus distinguish, within Nussloch loess units above tundra gley G4, eight additional slightly hydromorphic horizons with oxidized root tracks, hereafter called IGs, dated between about 27 and 21 ka (Fig. 3). By comparison with tundra gleys, IGs are thinner (only 10- to 15-cm thick) and have weaker iron redox imprints, implying a thinner permafrost active layer with lower ice content and weaker water release during the thawing season. Changes in mollusk assemblages recorded in IGs from Nussloch profile P3 also reflect weaker humidity increases as well as weaker or similar decreases in vegetation diversity but no appreciable warming phase (17) compared with tundra gleys. Likewise, magnetic properties of the sediment studied at high resolution throughout Nussloch profile P8 led to a similar conclusion (40). Although Nussloch tundra gleys are always associated with strong decreases in GSI and Greenland $[Ca^{2+}]$ (14), it is not systematic for IGs (Fig. 4). Moreover, no significant $\delta^{18}O$ increases in Greenland records can be identified in contemporaneous time intervals to IGs. The formation of these IGs may thus imply weaker precipitation increases and northward shifts of the Polar front than those leading to the formation of the main tundra gleys. The radiocarbon chronology established from the Nussloch loess sequence thus reveals a more complex pattern than identified until now within MIS 2 in Greenland $\delta^{18}O$ records. Indeed, the unsuspected centennial environmental variability exhibited in the Nussloch loess record during MIS 2 shows that the LGM was probably not as stable as generally admitted.

Among records that display short and low-magnitude oscillations during MIS 2, one can cite for the North Atlantic Ocean those of *Neogloboquadrina pachyderma* (sinistral) percentages from the Rockall Trough (41, 42) (Fig. 1), the Cariaco Basin reflectance influenced by movements of the Intertropical Convergence Zone (43), and the Bermuda Rise calcium concentration influenced by the Atlantic Meridional Overturning Circulation (44) (Fig. 4). In Europe, both Les Echets Lake magnetic susceptibility (45) and Eifel maar varve thickness (46) were likely influenced by the Aeolian transport, and in China, the Qinghai Lake dust flux above 25 μm was influenced by the Westerlies (47). The acquisition of more high-resolution and well-dated terrestrial and marine records is thus required to thoroughly evaluate the significance of both IGs and minor LGM oscillations. Additional development of coupled modeling experiments will be necessary to improve our understanding of associated climate mechanisms and interactions.

Future applications of this radiocarbon dating strategy based on earthworm calcite granules will improve correlations between loess sequences. This fundamental step forward will contribute to build a well-dated reference environmental framework across the European Loess Belt for the study of late Neanderthal and early Anatomically Modern Human peopling of Europe and their interactions with climate and environmental changes.

Methods

The Choice of Earthworm Calcite Granules for Radiocarbon Dating. West European Last Glacial loess sequences generally lack organic remains, such as wood, charcoals, and bones, which are required for radiocarbon dating. In this area, only particular geomorphological structures, like the Nussloch thermokarst infillings (19, 23) (Fig. S1 and Table S1), or archeological levels, may provide these materials that only exceptionally yield long-term coherent chronologies as, for example, in Central Europe (48, 49). A ubiquitous carbon-bearing material should thus be identified. To address the issue of a detailed chronology for the Last Glacial, the dating of loess bulk organic matter has been used throughout the Nussloch loess sequence (50). Despite a better precision than for OSL ages, these results show age underestimations

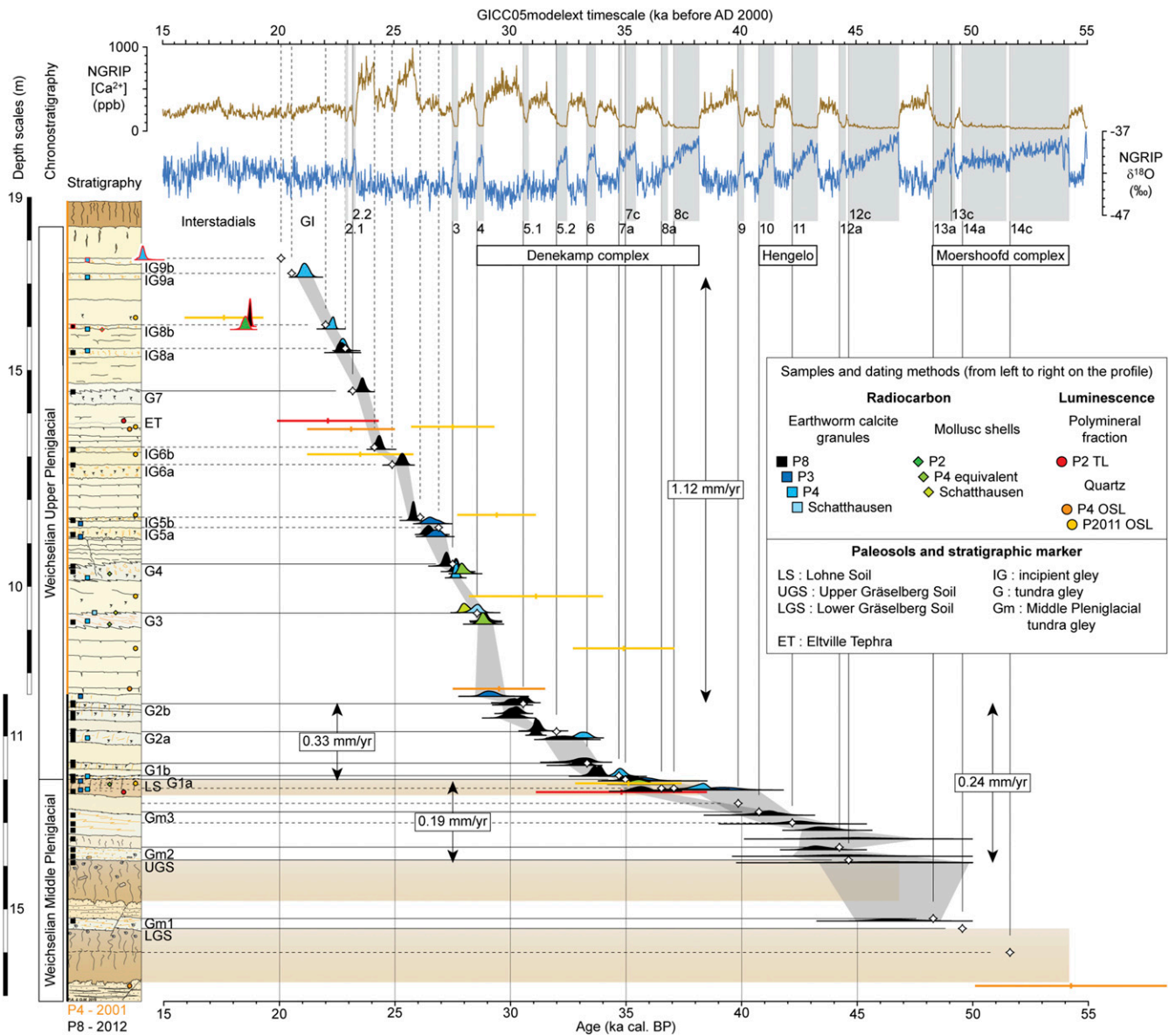


Fig. 3. Radiocarbon chronology of all soil horizons from the Nussloch P4–P8 composite loess profile based on earthworm granules and correlations with GIs. Discarded ages are red-contoured. The gray shading indicates 2σ ranges of ^{14}C ages. Dashed lines highlight IGs and contemporaneous minor decreases in Greenland $[\text{Ca}^{2+}]$ records (24). [Table S1](#) shows values and references of all displayed ages. TL, thermoluminescence.

and inversions, especially for tundra gley horizons (51). In addition, radiocarbon ages are, like feldspar OSL ages (14, 52, 53), significantly younger than quartz OSL and thermoluminescence ages (54–56). Alternatively, radiocarbon dating of terrestrial mollusk shells has been occasionally attempted from loess deposits (57–59). However, their results have always been criticized because of the possible incorporation of an undetermined fraction of old carbon in the shells (60). Despite this problem and the possible pollution by modern carbonates that could alter age accuracy, promising results have recently been obtained from several minute taxa from North American Quaternary deposits (61) and for the Nussloch P4 section (Germany) (53). In contrast, the few dates obtained from calcified root cells indicated that their formation can be significantly younger than the deposition of the surrounding sediment (62).

These results led us to look for another ^{14}C dating support for loess sequences. In this context, we noted the presence of numerous small calcite granules in the sieving residues resulting from the malacological study of the Nussloch P3 profile, especially in tundra gley horizons and arctic to boreal brown soils. It was then decided to test their chronological potential.

Characteristics of Earthworm Calcite Granules. Earthworm calcite granules generally have an ovoid form, ranging in size from 0.01 to 2.5 mm and, are

composed of aggregated sparite crystals organized in a radial structure achieved before their excretion (63) (Fig. 2). They are produced in Morren's glands that are laterally connected to the earthworm esophagus. Fixed by enzymatic reactions in these glands (64), calcium carbonate carbon originates mostly from feeding, essentially from litter and very few from humus and breathed atmospheric CO_2 (21). In addition, contributions in old and dead mineral carbon from soils are also extremely weak by *L. terrestris* (21), which limits any eventual artificial aging of dated granules.

Autopsies and breeding experiments revealed that earthworms of the genus *Lumbricus* are the largest granule producers among the most common European taxa. Selecting granules larger than 0.5 mm (22), which only *Lumbricus* taxa are able to produce, restrains any variability that could result from a multigenus material. Moreover, because the quantity of granules produced by *Lumbricus* species and excreted in the litter exceeds by one to two orders of magnitude the quantity excreted in the top 10 cm of the soil (22), they are then contemporaneous from the vegetation growing on the surface of the soil in which earthworms lived.

High-resolution counts recently undertaken throughout French Upper Weichselian loess sequences revealed very high *Lumbricus* granule abundances in tundra gley horizons and arctic brown soils (several hundred per 10 L of

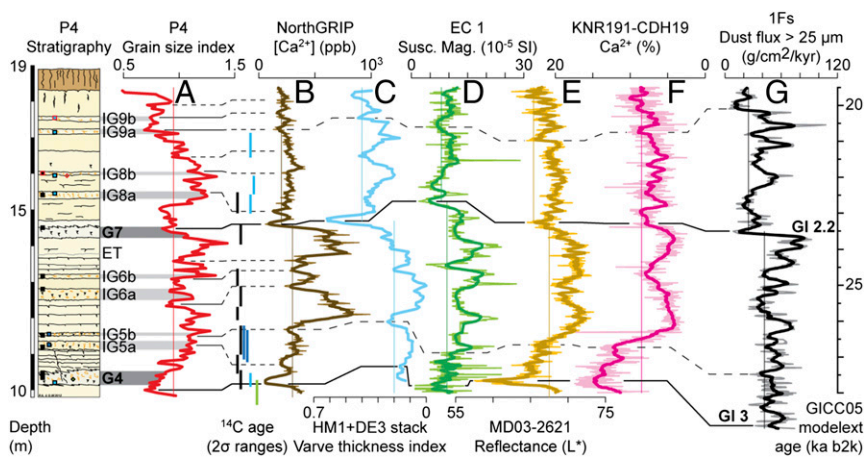


Fig. 4. Correlation attempt of Nussloch pedostratigraphy and GSI record with several different Northern Hemisphere paleoclimate records from 28 to 19.5 ka b2k (before AD 2000). (A) Nussloch P4 (14); (B) NorthGRIP (24) plus 5 samples moving average signals; (C) Eifel maar (46); (D–F) Les Echets Lake (45), Cariaco Basin (43), and Bermuda Rise (44) plus 21 samples moving average signals; and (G) Qinghai Lake (47) plus 11 samples moving average signals. Solid lines highlight established correlations between tundra gleys and interstadials. Short dashed lines highlight tentative correlations between Nussloch and other record.

sediment) and very low to zero abundances in loess units (20). Abrupt shifts in granule concentration associated with these stratigraphic transitions confirm their very low vertical dispersion. Moreover, in Last Glacial tundra gley horizons, which correspond to past permafrost active layers, earthworms were confined to the 0.2- to 0.4-m uppermost part of the soil profile that was unfrozen during the warm season. This restriction of their distribution prevented the creatures from any ingestion of old carbon from frozen underlying deposits. In addition, the absence of earthworm galleries and hibernation chambers in both tundra gleys and arctic brown soils suggests a strongly reduced burrowing activity in these layers, resulting from both superficial distribution during the warm season and feeding strategy in litter of *Lumbricus* species during milder and more humid seasons, like spring and autumn (25). The very low organic carbon content in loess (~0.1% wt), as in boreal and arctic brown soils (~0.4% wt), also strongly limits all eventual contribution of old or dead carbon in granules. Finally, the scarcity or absence of granules in pure loess units precludes any pollution of immediately underlying tundra gleys by younger fossil granules. In tundra gleys and arctic brown soils, high concentration in granules also allows for the sampling of thin sediment slices down to only 5-cm thick and including up to several hundred granules.

All of these criteria, therefore, favor the determination of accurate and precise ^{14}C ages from granules. However, the few trials of ^{14}C dating of granules (57, 62, 65, 66) remained insufficient to show their reliability in loess contexts. To provide a reliable chronology of continental interstadials, we decided to focus on the Nussloch loess–paleosol sequence that presents the best stratigraphic resolution for the Last Glacial in Western Europe (14, 23) (SI Text).

Acquisition of Modern and Fossil Earthworm Calcite Granules. In vitro breeding experiments have been conducted for 1 mo under controlled temperature (12-h diurnal cycle fluctuating between 14 °C and 16 °C with light) in plastic containers filled in with granule-free breeding ground; 20 g of calcium carbonate has been added to one of the containers. At the end of the experiments, granules have been collected from the uppermost 1–2 cm of experimental soil.

Fossil granules have been extracted from mollusk samples sieving residues from Nussloch P3, P4, and P8 profiles located along the axis of the same loess dune or “greda” (14, 23). Samples for terrestrial mollusks are systematically 10-L large, but those of the P8 profile were 5-cm thick and therefore, twice as thin as those of P3 and P4 profiles. All modern and fossil samples have been wet sieved on a 425- μm mesh.

Breeding experiments led on eight common European taxa revealed that only species from the *Lumbricus* genus are able to produce granules larger than 0.5 mm (22). Owing to the large granule abundance in selected samples, the use of a 0.8-mm mesh was sufficient to collect enough material for dating purpose. All granules have been hand-picked under binocular microscope with an initial optical selection of the cleanest ones and then washed for 10 min in distilled water in an ultrasonic tank to remove all eventual surface pollution (clay, iron oxides, and organomineral coatings). After drying, we selected under binocular 50 granules among the largest and visually cleanest to reach a minimum mass of 50 mg for each sample.

Radiocarbon Dating Protocol of Earthworm Calcite Granules. Granules were then slightly crushed in an agate mortar. All samples were leached with 0.01 M HNO_3 at room temperature for at least 30 min and rinsed with Milli-Q water to remove superficial contamination and oxidize any remaining organic matter. Extra water is removed using a Pasteur pipette.

At Laboratoire de Mesure du Carbone 14, samples were introduced into the bottom of a two-fingers reactor (67) and 1 cm^3 of pure H_3PO_4 (100%; previously distilled for 3 d at 105 °C and stored under argon) is added into the lateral reservoir. The reactor with the wet sample and H_3PO_4 is rapidly connected to the semiautomated vacuum line. The sample is then dried on the line, and the reactor is manually rotated to pour H_3PO_4 onto the samples (67).

At the Poznan Radiocarbon Laboratory, samples are introduced into a vial and dried under vacuum. Argon is introduced into the vial (up to overpressure). Vial is opened for a while just to introduce H_3PO_4 into the side arm of it. Argon is pumped out, and H_3PO_4 is poured onto the sample just afterward. Subsequent steps are similar in both laboratories: CO_2 evolving, water elimination, and evaluation of C quantity. CO_2 reduction and ^{14}C activity measurements are performed in Saclay (67, 68) and Poznan (69, 70) according to their respective protocols and equipment.

Check of Method Reliability. Radiocarbon dating of granules produced in laboratory breeding with and without addition of calcium carbonate yielded modern ages [101.8 ± 0.3 and 101.7 ± 0.4 pMC (percent modern carbon), respectively]. Thus, despite an age of $48,330 \pm 2,370$ B.P. (i.e., 0.24 ± 0.07 pMC) for the added calcium carbonate, both granule samples yielded ages similar to that of the soil organic matter from the breeding (270 ± 30 B.P.; i.e., 96.0 ± 0.3 pMC). Contribution in dead carbon of soil carbonates is undetectable and thus, negligible as previously concluded from isotope studies (21).

Probability distributions of calibrated granule-based radiocarbon ages have been generated using CALIB 7.0.2. software based on the IntCal13 calibration curve (71).

Comparisons of Nussloch and Greenland Chronologies. Because pedostratigraphic units result from a variable combination of weathering (downward) and Aeolian sedimentation (upward) processes, linking their lower and upper limits with onsets and offsets of GI is still not straightforward. The 2σ ranges of 35 calibrated ^{14}C ages from the main soil horizons have thus been compared with time intervals of the correlated GI. The maximum counting errors (MCEs) of the GICC05modelext age model (24), regarded as 2σ uncertainties, provide uncertainties on GI onsets and offsets. Extended GI time intervals at 1σ and 2σ have thus been calculated with one-half and full MCE values, respectively. All ^{14}C ages present an overlap with their associated GI 2σ intervals, and two ages from tundra gley G1a and G2a miss the overlap with GI 1σ intervals by only 87 and 134 y, respectively (Fig. S3). Most of ^{14}C ages present an overlap of 100% with GI 2σ intervals and above 60% for others. Also, 75% of the ^{14}C ages present an overlap higher than 50% with GI 1σ intervals, and for 90% of them, the overlap is higher than 35%. The distributions of these overlap values vs. the median value of the calibrated ^{14}C age show no particular trend.

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