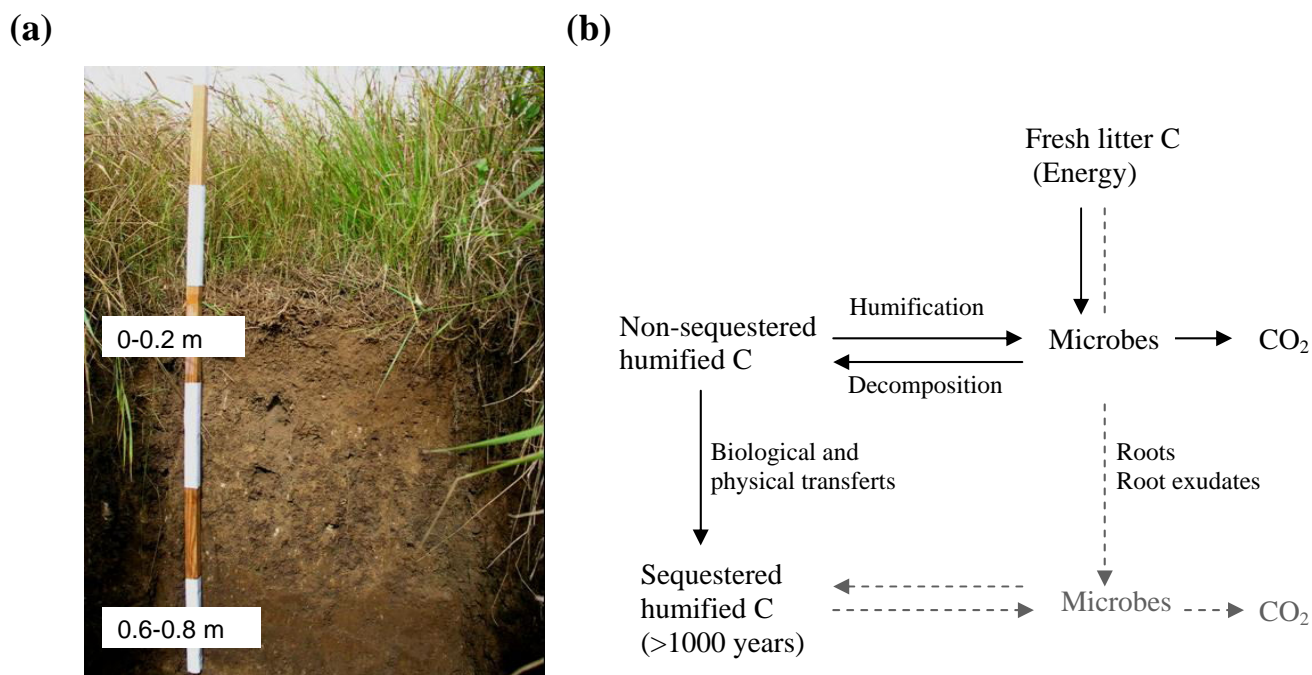


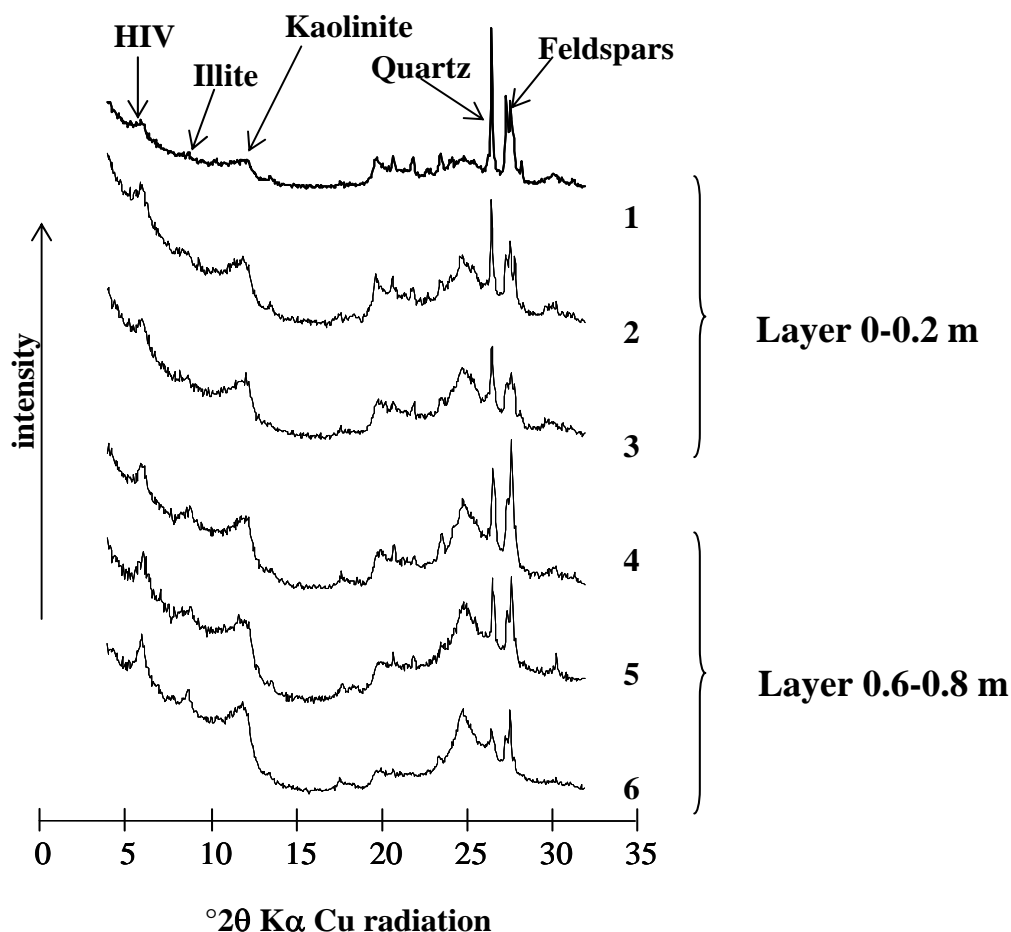
## SUPPLEMENTARY INFORMATION



**Supplementary Figure S1.** Picture of the studied profile (a), and suggested mechanisms of long-term carbon storage in deep soil layers (b). The soil profile was obtained by digging a pit of 3 x 1.5 x 1.2 m (length, width, depth). The two contrasted soil layers we studied are located within the A/B pedologic horizon for the surface layer (0-0.2 m) and the Bh horizon for the subsoil (0.6-0.8 m)(USDA nomenclature).

*Mechanisms of long-term carbon storage in deep soil layers.* Soil organic carbon (SOC) is the result of long-term accumulation of recalcitrant humified C compounds. In the surface layer (0-0.2 m), the supply of fresh-C by plants (litters and exudates) enables soil microbes to degrade these recalcitrant C compounds with their enzymes. The benefit of decomposing these recalcitrant compounds for microbial biomass lies in the concomitant release of nutrients (nitrogen, phosphorus). As a result, surface SOC is continuously recycled and C storage depends on the balance between decomposition and humification. According to this model, changes in climate, vegetation or land use may quickly (within a few years) disrupt this balance indicating that carbon stored in surface layer is vulnerable<sup>21</sup>. However, SOC

compounds becomes stored over long-time scale (>1000 years) when they are transferred to deep soil layers because the acquisition of energy from such substrates cannot sustain microbial activity and because the availability of fresh-C is typically low at depth. Various processes are involved in the burial of humus, namely, the illuviation of organic materials, the lixiviation of organo-metallic complexes, the displacement and accumulation of the soil matrix in depressed areas and the incorporation by earthworms and termites<sup>31</sup>. Any biological or physical factors favouring such a transfer towards deeper soil layers increase the storage of carbon over long-time scales in a stable compartment.



**Supplementary Figure S2.** Diffractograms of the clay fractions extracted from the six soil samples (2 soil layers, 3 replicates). Oriented preparations were analysed with a Phillips diffractometer using Cu radiation. The XRD were collected at  $0.05^{\circ}$  steps for 3 seconds

counting time in the range of 4 to 33° 2 $\theta$ . The X-ray patterns do not change markedly with depth. The six patterns exhibit clear feldspars and quartz peaks. There are also three peaks in the d(001) clay mineral range: the first at 6.2 °2 $\theta$  corresponding to the hydroxy-interlayered vermiculite minerals (HIV), the second at 8.8 °2 $\theta$  corresponding to the illite minerals and the third at 12.2 °2 $\theta$  corresponding to the kaolinite minerals. Overall, clay minerals in the two soil layers (kaolinite, HIV, illite) have a relatively low cationic exchange capacity and therefore capacity to retain associated C, compared to clays like montmorillonite and smectite that were absent<sup>15</sup>.

**Supplementary Table S1.** Soil organic carbon (SOC) content, storage and vertical distribution. Values are means  $\pm$ SE.

Soil layers	SOC content (g C kg <sup>-1</sup> )	SOC storage (kg C /m <sup>2</sup> )
0-0.2m	31.9 $\pm$ 0.7	5.6 $\pm$ 0.1
0.2-0.4m	29.4 $\pm$ 1.1	5.6 $\pm$ 0.2
0.4-0.6m	23.9 $\pm$ 1.1	4.3 $\pm$ 0.2
0.6-0.8m	23.3 $\pm$ 0.4	4.5 $\pm$ 0.1
0.8-1m	21.3 $\pm$ 0.0	4 $\pm$ 0.0
<b>TOTAL</b>		<b>24</b>

**Supplementary Table S2.** Percentage of chemical groups obtained by the integration of the NMR spectra of both soil layers. Values are given as means  $\pm$  SE (n=3). ns=not significant at P=0.05. The significance of change was tested for each chemical group by ANOVA. The percentages were (arcsine square root) transformed prior to analysis to conform with the assumption of normality.

Chemical groups	Layer 0-0.2m	Layer 0.6-0.8m	Significance of change
O-alkyl C	47.3 ±0.4	48.3 ±0.4	ns
Alkyl C	24.3 ±0.4	23.3 ±0.4	ns
Carboxylic C	16.3 ±0.4	14.3 ±0.8	ns
C substituted aryl C	8.7 ±0.4	10 ±0.0	P<0.02
O substituted aryl C	3.3 ±0.4	4 ±0.0	ns

**Supplementary Data 1.** Chemical analysis of SOC by Fourier-Transform Infrared spectroscopy (FT-IR).

Soil samples were treated with 10% hydrofluoric acid before the FT-IR analysis in order to remove mineral material. The FT-IR spectra were recorded with a Bruker tensor 27. We used the KBr technique (2 mg sample in 150 mg KBr) to obtain the absorption by SOC solid samples.

The FT-IR spectra of both soil layers (see below) are characterized by a strong peak ( $3405\text{ cm}^{-1}$ ) due to O-H stretching vibrations (included in alcohols, phenols and water molecules). This band is not considered in the following interpretation because it is strongly influenced by soil water content<sup>32</sup>. Other important band ( $1658\text{-}1652\text{ cm}^{-1}$ ) due to C=O stretching can be ascribed to amide groups. The broad band centred around  $1040\text{ cm}^{-1}$  due to C-O stretching may be assigned to aromatic esters and polysaccharides. Finally, small signals at  $2925\text{-}2927$  and  $2856\text{-}2858\text{ cm}^{-1}$  due to C-H stretching correspond to alkyl compounds<sup>33</sup>.

The intensity of the absorption bands depends on the relative amount of the absorbing groups<sup>34</sup>. Thus, we calculated the peak area of the absorbing groups and the corresponding percentages relative to total peak area in order to determine whether the proportion of absorbing groups changed with depth, that is, whether the chemical composition of SOC changed with depth. We precise here that the relative area percentage of absorbing groups

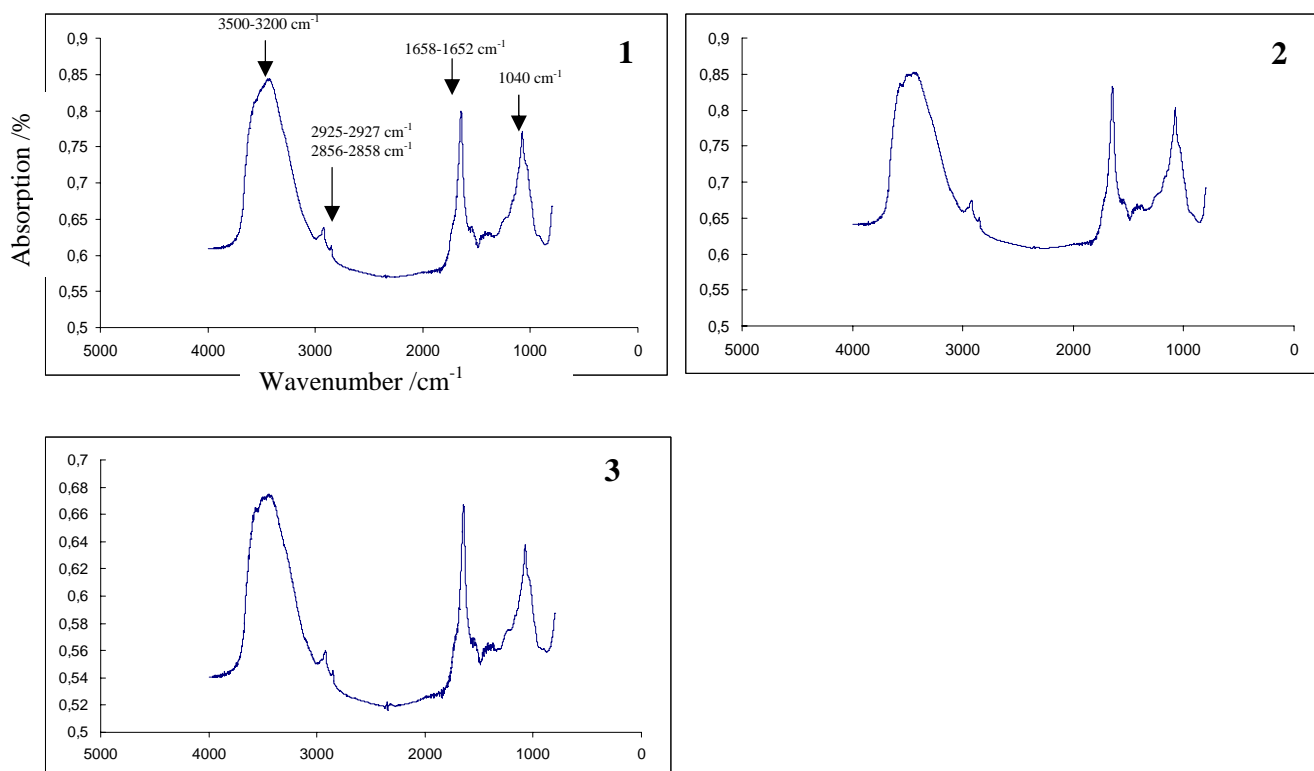
does not reflect their real contribution to soil organic matter<sup>34</sup>. Table 1 shows that the relative proportion of chemical groups identified with the FT-IR does not change significantly. Thus, FT-IR and NMR, which are complementary methods, both support the idea that the chemical composition of SOC does not change markedly with depth.

Table. Area percentages of the three absorbing groups identified by the FT-IR. Values are means  $\pm$ SE. ns=not significant at  $P=0.05$  (ANOVA). The percentages were (arcsine square root) transformed prior to analysis to conform with the assumption of normality.

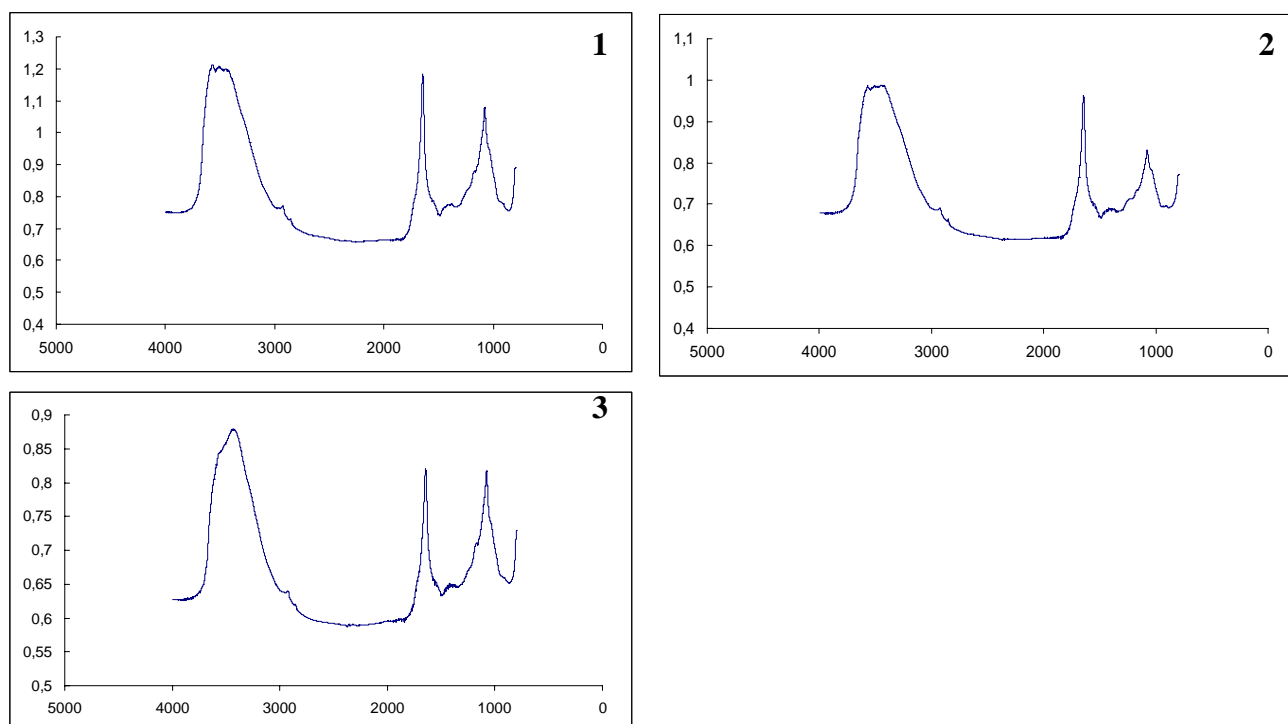
Absorbing groups	Layer 0-0.2m	Layer 0.6-0.8m	Signification of change
C-O stretching	3.7 $\pm$ 0.2	2.8 $\pm$ 0.4	Ns
C=O stretching	48.3 $\pm$ 2	48.7 $\pm$ 6	Ns
C-O stretching	48 $\pm$ 2	48.5 $\pm$ 6	Ns

The FT-IR spectra from the six soil samples (2 soil layers, 3 replicates)

### Layer 0-0.2 m



## Layer 0.6-0.8 m



**Supplementary Data 2.** Mean residence time (MRT) of particulate organic matter (POM) in the deep soil: 6.4 or 101 years?

The  $^{14}\text{C}$  model yielded two possible MRT<sup>25</sup> for POM of the subsoil,  $6.4 \pm 4.1$  or  $101 \pm 35$  years. However, the latter MRT is not consistent with our measurement of root density and POM content of the subsoil. Given a root density of  $0.008 \pm 0.002 \text{ g C kg}^{-1}$  and a MRT of 0.9 year for root tissue (this MRT has been calculated for the same grassland exposed to  $^{13}\text{C}$  labelled  $\text{CO}_2$ <sup>30</sup>), we estimated that the flux of fresh POM in the deep soil is  $0.009 \pm 0.002 \text{ g C kg}^{-1} \text{ year}^{-1}$  (Table 1). Thus, assuming equilibrium for POM pool and considering the POM content of the deep soil ( $0.016 \pm 0.05 \text{ g C kg}^{-1}$ ), we calculated that the MRT of POM should be  $(\text{POM content})/(\text{fresh POM flux}) = 2 \pm 0.8$  years which is consistent with the first MRT calculated by the  $^{14}\text{C}$  model.

**Supplementary Method 1.** Can the fixation of Cs on minerals explain the lack of Cs decomposition at depth? A modelling approach.

We used a simple model of SOC dynamics to test whether the fixation of SOC on minerals can explain the shift in mean residence time (MRT) of SOC with depth. The model considers two compartments, the free SOC compartment and the mineral-bound-SOC compartment, having distinct turnover rates ( $1/\text{MRT}$ ). The turnover rate of SOC in the soil surface ( $1/320 \text{ year}^{-1}$ ) and in the subsoil ( $1/2560 \text{ year}^{-1}$ ) is determined both by the distribution of SOC in the two compartments (proportion of SOC bound to minerals is 50% in the surface soil and 58% in the subsoil, Table 1) and by the turnover rate of these compartment. The turnover rate of free SOC ( $1/\text{MRT}_f \text{ year}^{-1}$ ) is assumed to be the same for both soil layers since the aim of the model is to test the role of minerals on SOC turnover time. The model reads as follows

$$\frac{1}{320} = 0.5 \frac{1}{\text{MRT}_f} + 0.5 \frac{1}{\text{MRT}_{bs}}$$

$$\frac{1}{2560} = 0.42 \frac{1}{\text{MRT}_f} + 0.58 \frac{1}{\text{MRT}_{bd}}$$

where  $\text{MRT}_{bs}$  is the MRT of SOC bound to surface minerals and  $\text{MRT}_{bd}$  the MRT of SOC bound to deep minerals. If we consider that the type of organo-mineral association does not change with depth, that is,  $\frac{1}{\text{MRT}_{bs}} = \frac{1}{\text{MRT}_{bd}}$ , then the solution of the model ( $\text{MRT}_f = 49.5$  years,  $\text{MRT}_{bs} = \text{MRT}_{bd} = -71.6$  years) has no physical meaning since  $\text{MRT}$  must have positive value. This indicates that the model and the fixation of SOC on minerals cannot explain the shift in MRT of SOC with depth. The only way to obtain positive solutions is to assume a large change in the type of organo-mineral association with depth, that is, organo-mineral complexes at depth must be 9.6 times more stable than in the surface layer ( $\text{MRT}_{bd} > 9.6 \times \text{MRT}_{bs}$ ). For example, if we consider  $\text{MRT}_{bd} = 10 \text{ MRT}_{bs}$ , the model can predict the shift in MRT of SOC with depth:  $\text{MRT}_f = 12,871$  years,  $\text{MRT}_{bs} = 162$  years and  $\text{MRT}_{bd} = 1,620$  years.

However, this assumption is not supported by our results. Table 1 and Supplementary Figure S2 showed that clay mineralogy, which was dominated by kaolinite, did not change markedly with depth. Fe and Al oxides and oxyhydroxydes, which play a role in the preservation of SOC, increased with depth (Fe x1.3, Al x1.2), but not to the extent imposed by our model (x9.6). Thus, the stability of SOC in the subsoil cannot entirely be ascribed to SOC fixation on minerals.

**Supplementary Method 2.** Details of the model<sup>25</sup> simulation estimating the MRT of SOC and POM from the <sup>14</sup>C analysis of SOC and POM.

The period of simulation (*p-b*) (see the model in Methods section) represented three times the length of the expected MRT<sup>25</sup>. The <sup>14</sup>C activity in the atmosphere was obtained from the literature<sup>35, 36</sup> for the period 1950-2003 and from modelling (simple exponential model) for years following 2003. <sup>14</sup>C activity in the atmosphere was assumed to be constant and equal to 100 pMC for the period before 1950. We assumed constant  $M_i$  and equilibrium for SOC pool.

However, given turnover times of SOC, it is likely that the inputs  $M_i$  and the SOC pool both fluctuated with time. We therefore studied the sensibility of our results to the assumption of constant  $M_i$  with two types of computer simulation. First, we simulated a random (uniform distribution) annual variation of  $M_i$  around 40% of its average to quantify the impact of the year-to-year fluctuation of primary production due to climate variability. Second, we tested the impact of a continuous change in primary production over several ten years that could be induced by a change in vegetation type (For example, a forest-to-grassland conversion). To this end, we increased or decreased  $M_i$  by 40% from 1956 to 2006. We choose this period because it corresponds to the period of bomb spike (maximising the impact of a change in  $M_i$ ) and because the vegetation of the site was possibly different before the 1950s'. The two types of simulation both showed that  $M_i$  has a limited bearing on the calculation of MRT. The



annual variation of  $M_i$  causes a  $1.7 \pm 0.6$  % (SE) MRT deviation from its correspondent constant  $M_i$  case for the surface layer, and a  $0.25 \pm 0.1$  % (SE) MRT deviation for the deep layer. The continuous variation of  $M_i$  causes a 23% MRT deviation for the surface layer, and a 2.6% MRT deviation for the deep layer. Thus, SOC turnover can be estimated without serious error by assuming constant  $M_i$ .

**Supplementary Notes.** Literature cited in the Supplementary Information.

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