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# Soil carbon storage and mineralization rates are affected by carbon inputs rather than physical disturbance: Evidence from a 47-year tillage experiment

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1 **Soil carbon storage and mineralization rates are affected by carbon**  
2 **inputs rather than physical disturbance: evidence from a 47-year**  
3 **tillage experiment**

4  
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24 AMG model

25

26 **Abstract**

27 In spite of the large number of studies conducted on the drivers of soil organic carbon (SOC)  
28 stocks, there is still no consensus on the impact of tillage on the distribution and turnover rate  
29 of SOC in the soil profile. Few studies have characterized precisely the turnover of SOC using  
30 <sup>13</sup>C natural tracing or simulated the SOC evolution per soil layer. In this study, we combined  
31 several approaches (diachronic analysis of SOC stocks, isotopic tracing and modelling) for  
32 characterizing the SOC evolution per soil layer in one of the oldest tillage experiments  
33 comparing no-till (NT), shallow till (ST) and full inversion tillage (FIT) combined with six crop  
34 managements. The new measurements made in 2017 reported in this paper confirm that i)  
35 tillage had no effect on SOC stocks integrated over the old ploughed layer (~0-28 cm) or  
36 deeper (~0-58 cm) and ii) reduced tillage affected the SOC distribution in the soil profile, with  
37 SOC storage in the upper layer (~0-10 cm) offset by a SOC loss in the underlying layer (~10-  
38 28 cm). The change in rotation (from C4 to C3 crops) in two crop management treatments  
39 allowed to quantify the decrease in C4 stocks during 19 years and calculate the specific  
40 mineralization rates relative to tillage treatments and soil layers. The mineralization rates did  
41 not vary significantly between tillage treatments in the whole (old) ploughed layer (~0-28 cm)  
42 but varied according to depth and tillage. The highest rates were found in the layer 0-5 cm of  
43 NT or ST and the lowest rates in the layer 10-28 cm of the same tillage treatments. The rates  
44 were highly correlated with estimated C inputs and particulate organic matter contents in each  
45 layer, but not with tillage intensity. The evolution of total SOC and C4 stocks of each soil layer  
46 was simulated with AMG model during 47 years. The standard model (with a single  
47 mineralization rate) gave a good prediction of SOC evolution when applied to the whole profile  
48 (~0-28 cm) but not for each individual layer. Including a relationship between C input and  
49 mineralization rate in the model allowed to well simulate the SOC evolution in all soil layers.  
50 This study shows that the main effect of a change in tillage on SOC storage is the change in  
51 the distribution of C input throughout the profile and the corresponding variation of the priming  
52 effect rather than the change in physical soil disturbance.

## 54 1. Introduction

55 The reduction and even suppression of tillage in arable soils has been proposed as one  
56 tool for enhancing carbon storage in soils leading to C sequestration (e.g. UNEP report, 2013).  
57 However, there is no consensus yet on the real impact of this technique. Powlson *et al.* (2014)  
58 indicated that “*the quantity of additional organic carbon in soil under no-till is relatively small*  
59 *and in large part an apparent increase resulting from an altered depth distribution*”. They  
60 pointed out that weaknesses in sampling methodologies, assumptions and interpretations in C  
61 sequestration studies comparing no-till (NT) to conventional tillage (CT) are causing  
62 overstatements of the potential of sequestering C in no-till soils.

63 Soil tillage may affect either the amount of C input in soil or the C output flux, *i.e.* the C  
64 mineralization rate. Both effects can occur. The total input of C may vary in relation with crop  
65 yield and crop biomass returned to soil including belowground (BG) materials. Crop yields have  
66 been shown to be comparable or slightly lower under NT than under CT (e.g. Pittelkow *et al.*,  
67 2016). In their meta-analysis, Virto *et al.* (2012) have shown that the variation in C input was a  
68 major factor explaining the variability in soil organic C storage due to tillage. But the main effect  
69 of tillage consists in altering the distribution of C inputs derived from aboveground (ABG) crop  
70 residues within the soil profile: no till systems receiving all ABG residues at soil surface  
71 whereas residues are mixed within tilled layers in tilled systems. The only sources of C input  
72 below soil surface in no-till systems are the root deposition and the transport of organic matter  
73 from the surface by bioturbation. Many studies have focused on the C stock in the upper soil  
74 layer (0-5 or 0-10 cm) under reduced tillage and few of them have analyzed the impact of the  
75 variation in C distribution within the profile.

76 The second possible effect of tillage is a modification in the C output flux. Six *et al.* (1999)  
77 suggested that NT management could result in a decrease of the decomposition rate of organic  
78 matter derived from original vegetation as one possible mechanism for increasing C  
79 sequestration. Several studies involving <sup>13</sup>C natural tracing suggested that the mean residence  
80 time (MRT, inverse of mineralization rate) could be higher under NT, probably due to an

81 increase in physical protection of organic matter (Balesdent *et al.*, 2000). However other  
82 studies (Murage *et al.*, 2007; Haile-Mariam *et al.*, 2008) found no difference in MRT between  
83 NT and CT systems. Therefore, no clear conclusion can be drawn up to now.

84 Methodological reasons can explain the discrepancies in results concerning C  
85 sequestration and turnover. A rigorous assessment of SOC stocks and SOC changes  
86 throughout time requires to follow five methodological recommendations: i) direct  
87 measurement of bulk density; ii) deep sampling, so that the sampling depth exceeds the  
88 maximum tilled depth (Luo *et al.*, 2010; Olson and Al-Kaisi, 2015); iii) calculation of stocks on  
89 an equivalent soil mass (ESM) and not on a depth basis (Wendt and Hauser, 2013); iv)  
90 pretreatment baseline measurement in the plots before treatments are applied (Olson, 2013)  
91 and v) use of a diachronic (*i.e. a time series analysis*) rather than a synchronic approach (Costa  
92 Junior *et al.*, 2013; Olson *et al.*, 2014). The latter authors indicated that “*to unequivocally*  
93 *demonstrate that the SOC sequestration has occurred at a specific site, a temporal increase*  
94 *must be documented relative to pretreatment SOC content*”. However, the diachronic analyses  
95 are rare (Dimassi *et al.*, 2014a).

96 Another source of divergence between published results concerns the evolution of SOC  
97 content below the upper soil layer (below 10 cm) under reduced or no-till (NT) compared to  
98 conventional tillage with ploughing (called HT or CT). The last meta-analysis made by Meurer  
99 *et al.* (2018) suggests that the mean difference NT-HT remains positive when the depth  
100 increases while the meta-analyses of Angers and Eriksen-Hamel (2008) and Luo *et al.* (2010)  
101 showed that the difference NT-CT becomes negative in soil layers between 15 and 40 cm.  
102 This divergence emphasizes the interest of a rigorous analysis of the SOC stocks in various  
103 layers and not simply in a single layer. It also suggests the interest of modelling the evolution  
104 of SOC stocks in various soil layers in order to test whether the main drivers of SOC evolution  
105 both at soil surface and below are well understood.

106 In this paper, we examine the effect of contrasted soil tillage and crop management on  
107 SOC evolution in a long-term experiment (LTE) which is one of the oldest tillage experiment

108 worldwide including a full diachronic analysis. We had three objectives: i) evaluate the  
109 consistency of the diachronic approach described by Dimassi *et al.* (2014a) for quantifying  
110 SOC storage after 47 years under contrasted tillage treatments; ii) characterize and  
111 disentangle the effects of C inputs and tillage operations on C mineralization rates in each soil  
112 layer; iii) evaluate the ability of the original or modified AMG model (Clivot *et al.*, 2019) to  
113 predict the evolution of SOC stocks in each soil layer. The originality of our approach consists  
114 in i) applying the methodological recommendations cited above, ii) following the SOC  
115 distribution throughout the soil profile and its evolution throughout time, ii) assessing our  
116 understanding of the SOC evolution through modelling.

117

## 118 **2. Materials and Methods**

### 119 **2.1. Experimental design**

120 The ongoing LTE on soil tillage, referred to as Experiment A, was established in 1970 at  
121 the experimental station of Arvalis at Boigneville in Northern France (48°19'37" N, 2°22'56" E).  
122 Details of the experiment can be found in Dimassi *et al.* (2014a) and Labreuche *et al.* (2018),  
123 including soil characteristics. The field is flat with a good internal drainage, so that no erosion  
124 takes place between plots or outside. The soil is a Haplic Luvisol [developed on loess and](#)  
125 [contains 24% clay, 65% silt and 9% sand](#). The average annual temperature, precipitation and  
126 potential evapotranspiration were 10.9°C, 627 and 736 mm over the whole study (1970-2017)  
127 and 11.7°C, 637 and 746 mm respectively during the last measurement period (2011-2017).

128 Three tillage treatments were established in 1970: no-till (NT), shallow tillage (ST) and full  
129 inversion tillage (FIT). In 1970, at the onset of the experiment, the soil was ploughed at a depth  
130 of *ca.* 28 cm. The ploughing depth was reduced progressively after 1970 to 25 cm down to 22  
131 cm in the last years. Mouldboard ploughing was realized in FIT every year at the end of  
132 autumn; superficial tillage (5-10 cm deep) was performed in ST every year either with rotavator  
133 or shallow mouldboard plough in order to favour crop residues decomposition. Seedbed  
134 preparation at sowing was similar in FIT and ST, and often consisted in two operations: rotary

135 harrow and tine harrow (0-5 cm depth). No tillage was practiced continuously in the NT  
136 treatment.

137 The layout consists in a randomized block system with four blocks. In addition to tillage  
138 treatments, six crop managements were established successively during four periods (Fig. 1).  
139 During the first period (1970-1982), all plots were cropped with a maize/winter wheat rotation  
140 and crop residues were chopped and spread at soil surface after harvest: this defines the first  
141 crop management (CM1) which was continued until 2017. During the second period (1982-  
142 1998), crop residues were removed from half of the plots creating a new crop management  
143 (CM6). Crop residues of CM6 were removed during the first 12 years (1982-1994) and returned  
144 again to soil in the following years. In 1998, half of the blocks were converted to a new 4-year  
145 rotation (winter wheat / barley / sugarbeet / pea) yielding two new crop managements without  
146 C4 crops (CM3 and CM4), CM3 deriving from CM1 and CM4 deriving from CM6. During the  
147 fourth period (2002-2017), plots corresponding to CM1 and CM6 were split into two subplots,  
148 half of them being managed as previously and the other half receiving a catch crop (oats/vetch)  
149 after wheat crop, yielding two crop managements: CM2 derived from CM1 and CM5 derived  
150 from CM6. All crops of the rotation were present every year in each crop management.  
151 Fertilization was similar in all tillage treatments. The mean N application rate was 175 kg ha<sup>-1</sup>  
152 yr<sup>-1</sup>.

153

## 154 **2.2. Soil sampling**

155 Soil sampling strategy was designed to calculate SOC stocks on ESM basis over a depth  
156 greater than the deepest tillage event ever made. Soil samples were collected in 1970, 1974,  
157 1978, 1982, 1986, 1990, 1994, 1998, 2002, 2007, 2011 and 2017. All details concerning the  
158 sampling methodology practiced until 2011 are given in Dimassi *et al.* (2014a). In 2017,  
159 sampling was realized with a hydraulic gauge ([Apageo, Magny, France](#)) to pull out intact soil  
160 cores of 6 cm diameter and 70 cm height. Each core was divided into seven subcores (layers  
161 0-5, 5-10, 10-15, 15-28, 28-33, 33-40 and 40-60 cm). Two soil cores were collected in each

162 plot and gathered together, giving 504 samples (3 tillage x 6 crop management treatments x 7  
163 layers x 4 replicates). Each sample was dried at 35°C and weighed in order to calculate its  
164 exact depth according to the measured bulk density.

165 Bulk densities were determined simultaneously in all plots over the whole profile, using a  
166 cylinder method for the upper 0-5 cm layer, a gamma densitometer (LPC-INRA, Angers,  
167 France) in the layers 5-40 cm (every 5 cm), and by weighing soil cores in the 40-60 cm layer.  
168 A total of 576 measurements was realized.

169

### 170 **2.3. Soil and plant analysis**

171 Coarse residues and roots present in the fresh soil cores were removed by handpicking.  
172 Soil samples were oven dried at 35°C for 96 hours, crushed to pass through a 2 mm sieve and  
173 finely ground with a ball mill (PM 400, Retsch, Germany). All soil samples since 1970 were  
174 analyzed for total carbon and nitrogen concentrations and <sup>13</sup>C abundance using an elemental  
175 analyzer (EURO EA, Eurovector, Milan, Italy) coupled to an isotope ratio mass spectrometer  
176 (Delta Plus Advantage, Thermo Electron, Germany). The presence of CaCO<sub>3</sub> was checked in  
177 all plots and layers. A few soil samples (essentially in the layer 40-60 cm) containing more than  
178 1 g CaCO<sub>3</sub> kg<sup>-1</sup> soil were decarbonated with a few drops of HCl 1M and re-analyzed for organic  
179 C.

180 Soil pH in water was measured for the different soil layers and treatments in 1982, 1994,  
181 2002, 2007 and 2017. Coarse particulate organic matter (CPOM) was determined on soil  
182 samples sampled between 1998 and 2011, as described by Autret *et al.* (2016). A 2 mm sieved  
183 and air dried soil sample of 50 g was dispersed under deionized water on a 200 µm sieve.  
184 Coarser particles (200–2000 µm) were washed out in a bucket during three minutes. Floating  
185 particles were collected (CPOM), oven dried at 60°C and finely ground with a ball mill. Their C  
186 concentration and <sup>13</sup>C abundance were determined as indicated previously.

187 Grain yields were determined every year in each plot as indicated by Dimassi *et al.*  
188 (2014a). ABG biomass was sampled at harvest in 2010, 2011 and 2012. Grain and straw



189 samples were analyzed to determine their carbon concentration and <sup>13</sup>C abundance using the  
190 same method than for soil samples.

191

#### 192 **2.4. Calculation of SOC and SOC-C4 stocks**

193 Calculations of SOC stocks were made at equivalent soil mass (ESM), using  
194 measurements of bulk density and organic carbon concentration. Comparing tillage treatments  
195 on ESM instead of soil depth basis is essential since no till results in changes in bulk density,  
196 with often lower bulk density in the 0-5 cm layer and increased bulk density in deeper layers  
197 (Powlson *et al.*, 2014). Calculations were made for six soil layers: L1 (700 t soil ha<sup>-1</sup>  
198 corresponding to ~ 0-5 cm); L2 (800 t ha<sup>-1</sup>, ~ 5-10 cm); L3 (800 t ha<sup>-1</sup>, ~ 10-15 cm); L4 (1760 t  
199 ha<sup>-1</sup>, ~ 15-28 cm); L5 (540 t ha<sup>-1</sup>, ~ 28-33 cm) and L6 (4000 t ha<sup>-1</sup>, ~ 33-60 cm). The reference  
200 soil mass in each layer was calculated using bulk densities measured in 1970 at the onset of  
201 the experiment. The reference mass in layer L1-4 (4060 t soil ha<sup>-1</sup>, corresponding to about 0-  
202 28 cm) includes and exceeds the deepest tillage event made during the experiment. Layer L6  
203 was sampled only in 2011 and 2017. All calculations made at ESM were realized using the  
204 method described in Autret *et al.* (2016) with a dedicated R package “SEME”, available on  
205 request (Mary *et al.*, 2018). Briefly, the soil is first discretized into elementary layers of 1 mm  
206 thickness. For a given plot and a given sampling year, a soil mass and a carbon concentration  
207 is assigned to each elementary layer *k*, depending on the measured layer to which it belongs.  
208 Then, the depth of SOC stock calculation (*z*) is determined each time to get as close as  
209 possible to the reference soil mass. Finally, the cumulative SOC stock *QC(z)* (in t C ha<sup>-1</sup>) in the  
210 layer 0-*z* is calculated as follows:

$$211 \quad QC(z) = 0.01 \sum_{k=1}^z \rho(k) \cdot C(k) \quad (1)$$

212 where  $\rho(k)$  and  $C(k)$  are respectively the bulk density (g cm<sup>-3</sup>) and the SOC concentration (g  
213 kg<sup>-1</sup>) of the elementary layer *k*.

214 At the start of the experiment, SOC mainly derived from C3 crops (wheat, barley, etc.) with  
215 a small proportion of C4 crops (maize). The maize-wheat rotation practiced in all treatments

216 lead to an increase in the SOC-C4 stock coming from the C4 crop (called thereafter SOC4).

217 This stock was calculated classically (e.g. Balesdent *et al.*, 1990) as follows:

$$218 \quad SOC4 = f \cdot SOC \quad (2)$$

$$219 \quad \text{with } f = \frac{\delta - \delta_3}{\delta_4 - \delta_3} \quad (3)$$

220 where  $f$  is the proportion of SOC derived from C4 plants,  $\delta$  is the  $\delta^{13}\text{C}$  signature of the SOC,  $\delta_3$   
221 and  $\delta_4$  are the  $\delta^{13}\text{C}$  signatures of the C inputs derived from C3 plants and C4 plants  
222 respectively. The values of  $\delta_3$  and  $\delta_4$  are taken as the average of all analyses of ABG plant  
223 organs ( $\delta_3 = -27.5 \text{‰}$  and  $\delta_4 = -12.5 \text{‰}$ ). Similar calculations were made with the CPOM stocks.

224

## 225 **2.5. Calculation of C mineralization rates**

226 In the treatments CM3 and CM4, the mixed C3-C4 rotation (maize-wheat) established  
227 from 1970 to 1998 was converted into a 4-year C3 rotation between 1998 and 2017. This  
228 resulted in an increase in SOC4 followed by a drop after 1998. The SOC4 decay could be fitted  
229 to an exponential function:

$$230 \quad SOC4 = (C_{04} - C_{s4}) \cdot \exp(-k \cdot t) + C_{s4} \quad (4)$$

231 where  $C_{04}$  is the initial SOC4 stock (in 1998),  $k$  is the mineralization rate (expressed per unit  
232 of SOC, in  $\text{yr}^{-1}$ ) of the  $SOC4$  stock and  $C_{s4}$  is an asymptotic value corresponding to the amount  
233 of stable carbon contained in the C4 stock. This function corresponds to equation (9) in  
234 Bernoux *et al.* (1998) who compared a single and double compartment model, and indicated  
235 that “when the derived systems are few decades old, it could be assumed that the more stable  
236 fractions are invariant over the period”. The amount of stable carbon was calculated by  
237 assuming that the percentage of stable carbon of C4 origin ( $C_{s4}/C_{04}$ ) in 1970 was similar in all  
238 layers and all treatments, since the soil of all plots had been mixed by annual ploughing until  
239 this date. This percentage was fixed at 35%, value which provided the best quality of fit. A  
240 sensitivity analysis of mineralization rates to this value was conducted.

241

## 242 **2.6. Statistical analysis**

243 All statistical analyses were performed using *R* (R Core Team, 2019). ANOVA was  
244 performed on C concentration, bulk density and SOC stock in each soil layer to evaluate the  
245 tillage and crop management effects in 2017. A linear mixed effect model was used with soil  
246 tillage and crop management as fixed factors and block as random factor. We used the *nlme*  
247 package (Pinheiro *et al.*, 2018) to fit the model. Significant differences ( $p < 0.05$ ) between  
248 tillage treatments were found using the *emmeans* function (Lenth, 2019). The assumptions of  
249 the linear mixed effect model were checked by visual examination of the residuals against  
250 predicted values and residuals histograms. Prior to SOC analysis, we used log transformed  
251 data or a Box-Cox transformation if necessary to meet assumptions of normality. The analysis  
252 was also applied to SOC stocks for layers L1-2, L3-5 and L1-5 at each sampling year from  
253 1970 to 2017. The mineralization rates obtained from the SOC4 stocks kinetics in 1998-2017  
254 for CM3 and CM4 treatments were analyzed using a second model, with tillage, crop  
255 management and layer as fixed factors and block as random factor.

256

## 257 **2.7. C inputs calculation**

258 Total C inputs (in layer L1-4) were calculated from crop yields as described by Clivot *et al.*  
259 (2019) using a methodology adapted from Bolinder *et al.* (2007). We used the measured data  
260 of dry matter yields and harvest indices to estimate ABG C inputs. We assumed that BG C  
261 inputs (root + extra-root C) were independent on crop yield but crop species dependent (see  
262 discussion in Clivot *et al.*, 2019). The annual BG C input per species was calculated using the  
263 average yield of the given crop species over all treatments and years and relative plant C  
264 allocation coefficients. These coefficients together with the humification rates of crop residues  
265 were obtained from Clivot *et al.* (2019).

266 The distribution of C inputs derived from ABG residues between layers L1 to L4 was  
267 calculated over the maximum depth of tillage by considering that all ABG residues were

268 distributed between the tilled layers proportionally to the soil mass of each layer, [assuming](#)  
269 [that ABG residues were homogenously mixed within tilled layers](#). The tillage depth considered  
270 was: 25 cm in 1970-1979, 24 cm in 1980-1989, 23 cm in 1990-1997 and 22 cm in 1998-2017  
271 for FIT; 10 cm in 1970-1998 and 5 cm in 1999-2017 for ST. ABG residues in treatment NT  
272 were supposed to enter layer L1 only. The distribution of C inputs derived from BG residues  
273 (roots + rhizodeposits) was first calculated using the asymptotic equation of Gale and Grigal  
274 (1987) and data retrieved from Fan *et al.* (2016) (see Clivot *et al.*, 2019 for more details). BG  
275 inputs distribution was then re-estimated for the tilled layers, assuming a distribution  
276 proportional to the soil mass, as for ABG inputs. The asymptotic root distribution was compared  
277 to the observed distributions reported by Qin *et al.* (2004, 2006): a very slight difference in root  
278 distribution was found between FIT and reduced tillage treatments (ST and NT). The C inputs  
279 calculated with the two methods were very close together and the asymptotic distribution was  
280 therefore used in the study.

281

## 282 **2.8. Simulation of SOC stocks with AMG model**

283 The evolution of SOC and SOC4 stocks were simulated using AMG model. [This model](#)  
284 [simulates soil C dynamics at an annual time step and considers three organic matter pools:](#)  
285 [fresh organic C coming from crop residues or organic amendments and SOC which is divided](#)  
286 [into active and stable pools \(Saffih-Hdadi and Mary, 2008\)](#). The last version of this model was  
287 successfully evaluated for predicting SOC evolution in a set of 20 long-term French  
288 experiments (Clivot *et al.*, 2019). The originality of our work consisted in simulating SOC  
289 evolution not only in the whole (old) ploughed layer (L1-4, corresponding to about 0-28 cm) but  
290 also in each individual layer (L1 to L4).

291 The mineralization rate of AMG model ( $k$ ) is the product of the potential mineralization rate  
292 ( $k_0$ ) and functions depending on soil characteristics (clay and  $\text{CaCO}_3$  contents, C/N ratio and  
293 pH) and climate (temperature and soil moisture conditions). The results of the present study  
294 indicated that it can also vary with the amount of C input (called  $I$ ). Regarding soil data inputs

295 for AMG, clay and CaCO<sub>3</sub> contents were considered invariant and homogeneous between  
 296 layers and treatments (239 and 0 g kg<sup>-1</sup> respectively). Soil pH and C:N ratio were defined for  
 297 each treatment, layer and year, according to the measurements and using linear interpolation  
 298 between measurement dates. We used the default values of the model for the SOC partitioning  
 299 in 1970: the initial proportion of the stable pool was set at 65% of total SOC and at 35% of total  
 300 SOC<sub>4</sub>. The vertical distribution of C inputs within the soil profile was assumed to be mainly  
 301 driven by tillage operations, the effects of bioturbation or liquid phase transport of organic  
 302 matter being considered negligible compared to tillage. AMG model was run in three steps:  
 303 In a first step, the model was used in its standard version. We used the standard parameters  
 304 of the soil mineralization model implemented in AMGv2 (Clivot *et al.*, 2019) including the  
 305 standard potential mineralization rate ( $k_0 = 0.29 \text{ yr}^{-1}$ ). In a second step, the mineralization rates  
 306  $k_{ij}$  determined in treatments CM3 and CM4 using the SOC<sub>4</sub> evolution during the period 1998-  
 307 2017 were forced in all treatments and over time. In a third step, the model was modified to  
 308 account for the relationship, called  $k_0(I)$ , found between mineralization rate and C inputs. The  
 309 modified version (AMGv3) calculates a potential mineralization rate specific of each layer,  
 310 tillage and crop management treatment depending on its C input. Two relationships were  
 311 tested: i) a linear function:

$$312 \quad k_0(I) = \alpha + \beta \cdot I \quad (5)$$

313 and an exponential function:

$$314 \quad k_0(I) = \alpha + (k_{00} - \alpha)(1 - \exp(-\beta \cdot I)) \quad (6)$$

315 where  $\alpha$  represents the mineralization rate in the absence of C input,  $\beta$  is a shape parameter  
 316 and  $k_{00}$  represents the asymptotic value of the mineralization rate.

317

### 318 **3. Results**

#### 319 **3.1. SOC stocks observed in 2017**

320 The organic C concentrations measured in 2017 and the corresponding SOC stocks are  
321 given in Table S1. They exhibit very similar trends with the previous observations made until  
322 2011 (Dimassi *et al.*, 2014a). SOC concentration varied little between crop management  
323 treatments and much more between tillage treatments, with no interaction between crop  
324 management and tillage. If we consider the mean of all crop management treatments (Table  
325 1), SOC concentrations in ST and NT treatments were significantly higher than in FIT for layers  
326 L1 and L2 and significantly lower for layers L3 and L4. No difference could be detected  
327 between tillage treatments in layers L5 and L6.

328 Bulk density was also affected by crop management and tillage, justifying the calculation  
329 of SOC stocks at equivalent soil mass (Table S2). Compared to FIT, bulk density in reduced  
330 tillage treatments was smaller near soil surface (0-10 cm), higher in 10-20 cm and lower below  
331 the old ploughing depth (< 25 cm).

332 Calculated SOC stocks varied widely with depth and tillage. Reduced tillage resulted in  
333 higher SOC stocks in layer L1-2 (~ 0-10 cm) and lower stocks in layer L3-4 (~ 10-28 cm). A  
334 full compensation occurred between layers since no significant difference in SOC stocks was  
335 found between tillage treatments over the old ploughed layer (L1-4) and even down to 60 cm  
336 (L1-6), whatever the crop management treatment. The mean SOC stock in the whole profile  
337 (L1-6) of all crop managements was  $65.2 \pm 4.9$ ,  $66.0 \pm 4.3$  and  $66.9 \pm 3.7 \text{ t ha}^{-1}$  for FIT, ST and  
338 NT, respectively.

339

### 340 **3.2. Evolution of SOC stocks from 1970 to 2017**

341 Fig. 2 shows the evolution of SOC from 1970 to 2017 in layers L1-2 (~ 0-10 cm), L3-5 (~  
342 10-33 cm) and L1-5 (~ 0-33 cm). It represents the mean of the six crop management treatments  
343 in view of the fact that no significant interaction was detected in SOC stocks between tillage  
344 and crop management (Dimassi *et al.*, 2014a). Layer L3-5 exceeds by about 5 cm the  
345 maximum depth ever tilled. In the FIT treatment, SOC stock in layers L1-2 and L3-5 increased  
346 very slightly with time. SOC content in layer L1-2 increased markedly and almost continuously

347 in the reduced tillage treatments (ST and NT). Simultaneously SOC stock in layer L3-5  
348 decreased almost continuously in these treatments. Compared to FIT treatment, the SOC  
349 content increased by  $5.5 \pm 0.8$  and  $5.5 \pm 0.9$  t C ha<sup>-1</sup> in layer L1-2 and decreased by  $-4.9 \pm 0.7$   
350 and  $-4.4 \pm 0.8$  t C ha<sup>-1</sup> in layer L3-5 in treatments ST and NT respectively, during the period  
351 1970-2017. When integrated over the two layers, *i.e.* below the maximum tillage depth (L1-5),  
352 the SOC stock followed exactly the same evolution in the three tillage treatments. The only  
353 significant difference which occurred in 1994 has been attributed to the climatic sequence  
354 (Dimassi *et al.*, 2014a).

355

### 356 **3.3 Evolution of SOC-C4 stocks after a change in the crop rotation**

357 In treatments CM3 and CM4, the change in rotation after 1998 from a mixed C3-C4 to a  
358 pure C3 crop rotation resulted in a marked change in the SOC4 dynamics (Fig. 3). The SOC4  
359 stock in the old ploughed layer (0-28 cm) increased from 1970 to 1998 during the maize-wheat  
360 rotation and then decreased during the following pure C3 rotation. The evolution was very  
361 similar in the three tillage treatments: the only difference was the slightly smaller increase in  
362 the treatment NT during the period 1978-1988. It is attributed to a slightly smaller maize yield  
363 and therefore a smaller amount of maize residues returned to soil. After 1998, the decline of  
364 SOC4 in the whole (old) ploughed layer was exponential and similar in all tillage treatments.

365 Conversely, the evolution of SOC4 stock in each layer (*i*) for each tillage (*j*) differed widely  
366 among layers and tillage treatments (not shown). Each kinetics observed from 1998 to 2017  
367 was fitted to the exponential model presented earlier (Eq. 4). The mineralization rates (called  
368 *kij*) thus obtained and the corresponding indicator of the quality of fit (relative RMSE) are given  
369 in Table 2. No significant difference was detected between treatments CM3 and CM4, so that  
370 they were considered together. The statistical analysis revealed that *kij* did not differ between  
371 layers under FIT, but differed in the reduced tillage treatments: it was about 50% higher in the  
372 surface layer (L1) than in the deeper layers (L2, L3 and L4). *kij* varied between tillage  
373 treatments, particularly in the upper layer: the mineralization rate in L1 was much higher in ST

374 and NT (0.133 and 0.125 yr<sup>-1</sup> respectively) than in the FIT treatment (0.081 yr<sup>-1</sup>). This  
375 conclusion remains valid even if we change the parameter  $C_{s4}$ , *i.e.* the initial amount of stable  
376 carbon contained in the SOC4 stock. The sensitivity analysis showed that the differences  
377 between treatments were similar to those obtained with the standard value of  $C_{s4}$  (Table S4).  
378 It is noticeable that the mineralization rates, calculated for the whole (old) ploughed layer (L1-  
379 4, ~0-28 cm), were absolutely similar ( $0.080 \pm 0.001$  yr<sup>-1</sup>) in the three tillage treatments. This  
380 result is also consistent with the evolution of the C4 content of particulate organic matter. The  
381 mineralization rate of the POM-C4 stock in layer L1-4 did not differ between tillage and crop  
382 management treatments (Fig. S1). Its mean value among tillage treatments was  $0.42 \pm 0.03$   
383 yr<sup>-1</sup>.

384

### 385 **3.4 Mineralization rates and C inputs per soil layer**

386 The mineralization rates were then compared to the C inputs ( $C_{ij}$ ) calculated for each soil  
387 layer ( $i$ ) and each tillage treatment ( $j$ ). The C inputs were calculated annually and then  
388 averaged over two periods. Estimated total C inputs in the whole (old) ploughed layer (L1-4)  
389 increased from  $3.04 \pm 0.02$  to  $4.06 \pm 0.06$  t C ha<sup>-1</sup> yr<sup>-1</sup> between the first (1970-1998) and the  
390 second period (1998-2017), due to the increase in crop biomass production (Table 3 and S3).  
391 The total C inputs were very close between tillage treatments, as previously found for crop  
392 yields (Dimassi *et al.*, 2014a), but the distribution within the profile was very different. For  
393 example, C inputs in layer L3 were higher than those in layer L1 in the FIT treatment but  
394 represented only 5% to 7% of the C inputs in layer L1 of treatment NT.

395 When considering the 4 layers and the 3 tillage treatments, we found a close relationship  
396 (Fig. 4) between the C mineralization rates ( $k_{ij}$ ) previously determined from the SOC4 stock  
397 dynamics and the C inputs ( $C_{ij}$ , expressed in g C kg<sup>-1</sup> soil) calculated during the same period  
398 (1998-2017). The regression line was  $k_{ij} = 0.015 C_{ij} + 0.060$  ( $r^2 = 0.93$ ;  $p < 0.001$ ). In fact, the  
399 relationship was even better described by a non-linear function, as follows:  $k_{ij} = 0.185 (1 -$   
400  $\exp(-0.102 C_{ij})) + 0.059$ . The interest of the latter function is to simulate an asymptotic increase



401 of the mineralization rate when C input increases (asymptotic value = 0.244 yr<sup>-1</sup>), as expected.  
402 We also found a close correlation between  $k_{ij}$  and the mean CPOM content ( $P$ , expressed in  
403 g C kg<sup>-1</sup> soil) measured over the period 1998-2011 (Fig. S2):  $k_{ij} = 0.067 P + 0.052$  ( $r^2 = 0.93$ ;  
404  $p < 0.001$ ). Such a relationship is not surprising since CPOM is expected to be a proxy for the  
405 amount of C input.

406

### 407 **3.5 Simulation of SOC stocks per soil layer with AMG model**

408 AMG model was run for each layer, tillage and crop management treatment (4 x 3 x 6 = 72  
409 situations) in order to simulate the evolution of SOC and SOC4 stocks. The simulations were  
410 run either with the standard potential mineralization rate ( $k_0$ , step 1) or the mineralization rates  
411 specific of each layer and tillage treatment determined previously ( $k_{ij}$ , step 2). The comparison  
412 between simulated and observed stocks during two periods (1970-1998 and 1998-2017) is  
413 presented at Fig. 5.

414 We first analyze the second period (1998-2017) which was used for calculating the decay of  
415 SOC4 stocks in treatments CM3 and CM4. During this period, the quality of fit in the FIT  
416 treatment was about the same between the two mineralization options: the standard ( $k_0$ ) and  
417 the specific one ( $k_{ij}$ ). This was expected because  $k_{ij}$  varied little among soil layers in this  
418 treatment. The SOC stocks were rather well simulated in all layers and SOC4 stocks were  
419 slightly underestimated in L4 for both options. Conversely, the model prediction of SOC and  
420 SOC4 stocks was markedly improved in treatments ST and NT when considering the specific  
421 mineralization rates which varied widely between soil layers (Table S5). For example, the mean  
422 RMSE for the SOC stock in layer L1 of NT treatment dropped from 4.70 t C ha<sup>-1</sup> with the  
423 standard mineralization rate to 1.22 t C ha<sup>-1</sup> with the specific mineralization rate. Therefore, the  
424 conclusions made in CM3 and CM4 were valid for the other treatments: the soil layers receiving  
425 higher C inputs had greater mineralization rates than those receiving low C inputs. Our results  
426 also demonstrate that mineralization rates were not related to tillage intensity, for the following  
427 reasons: i)  $k_{ij}$  were similar in layer 1 of treatments ST and NT although this layer was tilled in

428 ST but not NT; ii)  $k_{ij}$  in layer 1 was higher in ST than FIT although layer 1 was tilled in both  
429 treatments (and even more in the FIT treatment).

430 However, during the first period (1970-1998), the predictions with the specific mineralization  
431 rates were not better than those realized with the standard mineralization rate. In particular,  
432 SOC stocks in layer L1 of ST and NT treatments were underestimated with the  $k_{ij}$  model. In  
433 these treatments, the model error was larger for SOC stocks but smaller for SOC4 stocks.

434 If we consider both periods (1970-2017), the quality of fit was improved when using specific  
435 mineralization rates, particularly in the first layer of reduced tillage treatments (Table 4). For  
436 example, the mean RMSE in layer L1 of NT treatment was 4.15 t C ha<sup>-1</sup> with the  $k_0$   
437 mineralization rate and only 1.93 t C ha<sup>-1</sup> with the specific mineralization rate. The quality of  
438 prediction of SOC4 stocks was also improved with the specific mineralization rates: for  
439 instance, the RMSE in layer L1 of NT treatment decreased from 2.21 t C ha<sup>-1</sup> with  $k_0$  to 0.56 t  
440 C ha<sup>-1</sup> with  $k_{ij}$ .

441

### 442 **3.6 Evaluation of the new mineralization rate model**

443 The new mineralization rate model including equations (5) or (6) was implemented in AMG  
444 model (AMGv3) and compared to the standard version (AMGv2). AMGv3 assumes that the  
445 potential mineralization rate  $k_0$  increases when the C input increases, either linearly (Eq. 5) or  
446 exponentially (Eq. 6). The parameters  $\alpha$ ,  $\beta$  and  $k_{00}$  were fixed using the regression equation  
447 (Fig. 4). The quality of fit was similar using the linear or the exponential relationship. We  
448 therefore used only the exponential relationship for comparison with AMGv2. Increasing the C  
449 input from 1.0 to 4.0 g C kg<sup>-1</sup> soil yr<sup>-1</sup>, which corresponds approximately to the change which  
450 occurred after 1970 in layer L1 of treatments ST and NT (0.7 to 2.8 t C ha<sup>-1</sup> yr<sup>-1</sup>), should have  
451 almost doubled the potential mineralization rate, from 0.31 to 0.55 yr<sup>-1</sup>. Conversely, the  
452 reduction in C input from 1.0 to 0.2 g C kg<sup>-1</sup> soil yr<sup>-1</sup>, which occurred in layer 4 of the same  
453 treatments, would have slightly decreased the potential mineralization rate, from 0.31 to 0.23  
454 yr<sup>-1</sup>.

455 The new mineralization model AMGv3 could reproduce very satisfactorily the evolution of SOC  
456 stocks in each soil layer in the three tillage treatments (Fig. 6.) If we consider the whole dataset,  
457 the quality of fit was good (Table 4), as indicated by the **small MD and RMSE**, most often  
458 smaller than the standard deviations of measurements (mean SD = 0.80 t C ha<sup>-1</sup>). The quality  
459 of fit was better in the new model  $k_0(I)$  compared to the  $kij$  model (fixed values of Table 2) and  
460 better than the standard  $k_0$  model, since the average RMSE was 0.77, 0.93 and 1.26 t C ha<sup>-1</sup>  
461 respectively, and the mean **absolute MD** was 0.34, 0.55 and 0.84 t C ha<sup>-1</sup>. The new model  
462 greatly improved simulations in layer L1, which were poorly simulated by the standard  $k_0$   
463 model. **These results confirm that the observed decline in the SOC stock in layers L3 and L4**  
464 **of treatments ST and NT results mainly (if not exclusively) from the marked reduction in C**  
465 **inputs derived from ABG crop residues compared to FIT treatment or the initial situation.**

466 If we consider the total SOC stock in L1-4 (~0-28 cm), summing up individual simulations of  
467 layers L1 to L4, AMGv3 performed better than AMGv2 for ST and NT treatments (Fig. 7, Table  
468 4). Indeed, SOC stocks in these treatments were overestimated by AMGv2 after 1998.  
469 Furthermore, AMGv3 simulated a similar evolution of SOC stocks in the three tillage  
470 treatments, **consistently** with the observed data. Finally, when simulating directly the whole  
471 layer L1-4 (which is the default use of the model), both models gave similar results, close to  
472 the sum of individual simulations of layers L1 to L4 with AMGv3 (Fig.7).

473

## 474 **4. Discussion**

### 475 **4.1. Tillage effects on SOC storage in the long-term**

476 The diachronic analysis of SOC evolution in the soil profile of our LTE (47 years) confirms the  
477 results obtained in the same experiment (Dimassi *et al.*, 2014a) and in two other LTEs made  
478 on the same site (Dimassi *et al.*, 2013; Mary *et al.*, 2014): reduced tillage and even no-till did  
479 not result in permanent additional SOC storage compared to annual ploughing if SOC stocks  
480 are calculated over a depth equal or greater than the maximum tillage depth ever done. This  
481 conclusion is in line with the meta-analysis made by Luo *et al.* (2010) who selected

482 experiments with a depth greater than 40 cm. It was obtained under a rather wet and temperate  
483 climate and may not apply to situations where crop yields differ widely between tillage systems  
484 or under semi-arid conditions (e.g. Blanco-Moure *et al.*, 2013). Many recent papers, such as  
485 Dikgwatlhe *et al.* (2014), Powlson *et al.* (2014), Olson and Al-Kaisi (2015), Singh *et al.* (2015),  
486 Valboa *et al.* (2015), Piccoli *et al.* (2016), Fujisaki *et al.* (2017), Martinez *et al.* (2017) or Hiel  
487 *et al.* (2018), confirm that the reduced tillage has a major impact on SOC distribution in soil but  
488 may not change the total SOC content when a sufficient depth is considered.

489 One originality of this paper is to make a diachronic analysis per layer, which is rarely done in  
490 the literature. We found a continuous increase of SOC stock in the upper layer of the reduced  
491 tillage treatments but also a continuous loss of carbon in the deeper layers (10-33 cm) which  
492 have not reached yet their SOC equilibrium. In comparison with FIT, the mean rate of change  
493 in layer L3-4 (10-28 cm) of treatments ST and NT was  $-0.10 \pm 0.03$  and  $-0.09 \pm 0.04$  t C ha<sup>-1</sup>  
494 yr<sup>-1</sup> (mean and confidence interval of the slope of the linear regression) respectively,  
495 corresponding to a loss of 18% and 16% of initial soil carbon after 47 years. Comparable SOC  
496 losses can be calculated in two diachronic studies reported in Spain: Hernanz *et al.* (2009)  
497 found that SOC stock in 10-40 cm had decreased by 14% and 19% after 20 years in ST and  
498 NT treatments respectively; López-Fando and Pardo (2011) observed a SOC loss of 23% and  
499 31% respectively in the layer 10-30 cm after 16 years. The data of Clapp *et al.* (2000) from the  
500 Rosemount LTE (USA) shows a 7 to 30% decrease of SOC in 15-30 cm after 13 years. These  
501 high net decay rates (7-31%) challenge the commonly reported assumption that no-tillage  
502 reduces the C mineralization rate in the undisturbed soil layers, due to physical protection.

503

#### 504 **4.2. Mineralization rate estimates with <sup>13</sup>C tracing methodology**

505 The change in crop rotation (from mixed C4/C3 to exclusive C3 plants) realized in treatments  
506 CM3 and CM4 from 1998 to 2017 allowed us to calculate the mineralization rate of the soil  
507 organic matter derived from C4 plants grown from 1970 to 1998. We found that the SOC-C4  
508 stock declined in all layers according to a first order kinetics. The mineralization rate obtained

509 over the whole profile (0-28 cm) was remarkably identical in the three tillage treatments, with  
510 a mean value  $k_{ij} = 0.080 \pm 0.001 \text{ yr}^{-1}$ . This corresponds to a mean residence time of the active  
511 pool of  $12.5 \pm 0.1$  years. This result might appear contradictory with the literature. Two studies  
512 (Balesdent *et al.*, 1990; Six *et al.*, 1998), also using  $^{13}\text{C}$  natural abundance methodology,  
513 indicated that the MRT of SOC under no tillage was greater than that under full inversion tillage.  
514 However, two other studies based on the same technique obtained opposite conclusions.  
515 Murage *et al.* (2007) found that the turnover of SOC-C3 in the old ploughed layer of an 11-yr  
516 experiment in Canada was unaffected by tillage (NT vs CT). Haile-Mariam *et al.* (2008) found  
517 no difference (on average) in MRT between no-till and tilled systems in three LTEs in USA,  
518 both *in situ* and in laboratory incubations. However, these studies have serious flaws: i) they  
519 considered only two dates, an initial and a final point, without providing a diachronic follow-up  
520 which reduces uncertainties due to spatial heterogeneity of soils (Fujisaki *et al.*, 2017); ii) the  
521 initial stock (SOC and SOC4) was not or partially measured and assumptions had to be made  
522 to estimate it; iii) there was no statistical analysis of the differences between tillage treatments.  
523 Several papers have emphasized the importance of a full diachronic approach with sufficient  
524 data points over time to estimate MRT accurately (Bernoux *et al.*, 1998; Derrien and Amelung,  
525 2011).

526 Our results can be compared to those obtained in two incubation studies made previously in  
527 the same experiment (Oorts *et al.*, 2006; Dimassi *et al.*, 2014b). We calculated the  
528 mineralization rates relative to a reference, chosen as the layer L1 of the FIT treatment (Fig.  
529 8). Results show that the variations in mineralization rates observed *in situ* between layers and  
530 treatments are confirmed by the previous incubation studies, even though the mineralization  
531 rates determined *in situ* concern the SOC-C4 formed with maize crops grown before 1998 (at  
532 least 14 years from 1970 to 1998) while the mineralization rates calculated in incubation  
533 studies concern the whole SOC stock. In another experiment made on a similar soil type,  
534 Sauvadet *et al.* (2017) compared a reduced tillage (RT) and a full inversion tillage (CONV)

535 treatment in a 6-yr experiment. They incubated the upper soil layer (0-5 cm) and found that the  
536 specific mineralization rate of RT was 62% higher than that of CONV, confirming our results.

537

### 538 **4.3 Mineralization rates versus tillage and depth**

539 Our study also revealed that the *in situ* mineralization rate of SOC4 stock varied within layers  
540 and tillage treatments with a significant interaction between them. While no significant  
541 difference appeared between layers **under FIT**, differences were found in the reduced tillage  
542 treatments: the mineralization rate of the upper layer (L1, ~0-5 cm) in NT and ST was much  
543 higher than that observed in the FIT treatment and slightly smaller in lower layers (below 10  
544 cm). The absence of difference between layers **in FIT** was expected since the annual ploughing  
545 mixes the soil and crop residues, homogenizing almost completely their concentrations within  
546 the profile. The reduced mineralization rate in layers L3 and L4 of treatments ST and NT can  
547 be attributed to an absence of soil disturbance which could result in an increased physical  
548 protection (*e.g.* Balesdent *et al.*, 2000) or to smaller C inputs due to the absence of mechanical  
549 incorporation of ABG crop residues. It is not possible to disentangle the two processes in these  
550 layers. But the absence of difference in mineralization rates in layer L1 between treatments ST  
551 and NT, which received similar amounts of crop residues but strongly differed in tillage  
552 operations, and the lower mineralization rate in the same layer of the FIT treatment which was  
553 tilled intensively, indicate that the first hypothesis has a minor importance. The high correlation  
554 found between the mineralization rate and C inputs strongly suggests that the **main driver** of  
555 the mineralization rate is the intensity of C inputs and not the physical soil disturbance.

556

### 557 **4.4 Fresh C input as a determinant of C mineralization rate**

558 The amount of C input is known to be an important factor driving the changes in SOC stocks  
559 (*e.g.* Luo *et al.*, 2010; Powlson *et al.*, 2011; Li *et al.*, 2018), including the changes due to tillage  
560 (Virto *et al.*, 2012). In this study we show that, under field conditions, the amount of C input  
561 also drives the mineralization rate (per unit of SOC) of the stabilized organic matter. This

562 conclusion agrees with the results of several other studies. Duong *et al.* (2009) showed that a  
563 higher frequency of residue addition increased the C mineralization rate. Gude *et al.* (2012)  
564 found that the MRT of SOC was smaller in a high input site than in a low input one. Diochon  
565 *et al.* (2016) analyzed the results of a 17-yr LTE comparing a continuous maize, a soybean-  
566 maize rotation and a continuous fallow soil and observed that the mineralization rates of the  
567 SOC-C3 stocks were correlated with the mean C inputs. Using  $^{13}\text{C}$  enriched residues, Sarker  
568 *et al.* (2018) showed that crop residue input increased native SOC mineralization *via* positive  
569 priming. Cardinael *et al.* (2015) compared the evolution of SOC in a 52-yr LTE maintained bare  
570 fallow or receiving applications of wheat straw and found that mineralization rates were much  
571 lower in the bare fallow soil.

572 Therefore, the evolution of SOC is under the control of two opposite processes. The variation  
573 in the mineralization rate vs C input level is a feedback effect, which could offset the positive  
574 effect of increasing C inputs on SOC. In most cases such as in our study, the compensation is  
575 incomplete so that the general behavior is a positive correlation between C input and SOC  
576 stocks (*e.g.* Liu *et al.*, 2014; Li *et al.*, 2018). However, there are a few cases where the  
577 compensation can be very important, leading to an opposite relationship. This has been found  
578 in situations when N availability is very limiting, such as described by Fontaine *et al.* (2011) or  
579 Diochon *et al.* (2016).

580 The mechanism behind this offset is likely to be the priming effect (PE) due to the addition of  
581 fresh organic matter, which has been shown to be a ubiquitous process in all soils (Perveen *et al.*  
582 *et al.*, 2019). Several studies made with glucose, cellulose or straw addition indicated that PE  
583 increases with the C addition rate (Mary *et al.*, 1993; Guenet *et al.*, 2010; Paterson and Sim,  
584 2013; Liu *et al.*, 2017; Fang *et al.*, 2018; Shahzad *et al.*, 2019; Liu *et al.*, 2020), and probably  
585 until a saturation level which has not been identified because it interacts with the nutrient level  
586 in soil (Fontaine *et al.*, 2011; Dimassi *et al.*, 2014b). In this study, we found that the relationship  
587 can be considered to be about linear until a concentration of  $5 \text{ g C kg}^{-1}$ . Sauvadet *et al.* (2018)  
588 found that the PE was similar in the RT and FIT treatments for a given amount of C added,

589 suggesting that the priming is mainly driven by the C addition rate and not by the tillage  
590 practices.

591 On the basis of a laboratory incubation characterizing the PE in the Boigneville experiment in  
592 2012, Dimassi *et al.* (2014b) predicted that “*PE intensity should vary within the soil profile with*  
593 *a maximum in the upper soil layer of NT treatment and a minimum in the lower layer of the*  
594 *same treatment. The mineralization rate of SOM should vary similarly and its mean residence*  
595 *time in the opposite way, suggesting that SOM could reach steady state in upper layer of NT*  
596 *more rapidly than in FIT and even more than in the no-tilled layers.*” These predictions are  
597 confirmed by the present study.

598

#### 599 **4.5. Simulation of SOC dynamics using the new mineralization model AMGv3**

600 On the basis of the results obtained using the <sup>13</sup>C natural tracing technique, we could propose  
601 a new mineralization model which considers the soil and environmental factors already known  
602 to influence mineralization rates (Clivot *et al.*, 2017, 2019), but also the effect of C input  
603 intensity, itself attributed to the priming effect. Applying this model (called AMGv3) to our whole  
604 dataset allowed to simulate accurately SOC stocks in most situations, without considering any  
605 extra physical protection in the reduced tillage treatments. We conclude that the main effect of  
606 a change in tillage on SOC storage is the change in the distribution of C input throughout the  
607 profile and the corresponding variation of the PE rather than the change in physical soil  
608 disturbance. The exponential function  $k_0(l)$  that we propose looks like that proposed by Guenet  
609 *et al.* (2018) and allows simulating a saturation effect for high C inputs. It would be interesting  
610 to test the equation for grassland soils, which are not tilled and receive high amounts of C  
611 inputs, mainly through rhizodeposition.

612 Overall, the new model was able to reproduce satisfactorily the SOC dynamics in all individual  
613 layers and the whole (old) ploughed layer. We noticed that in the last periods (2002-2017) it  
614 slightly overestimated SOC stocks in layer L1 and underestimated them in layer L2 of the NT  
615 treatment. One possible explanation of these two differences could come from a biased



616 estimate of C input, which could result from transport processes (neglected here): downwards  
617 transport of SOC due to bioturbation or liquid phase transport. These processes are difficult to  
618 quantify. Including formalisms for these processes (Braakhekke *et al.*, 2011; Keyvanshokouhi  
619 *et al.*, 2019) might further improve the model performance. However, the effects of these  
620 processes are expected to be small. In a no-till experiment, Jha *et al.* (2017) compared 6  
621 treatments [varying in maize addition rate](#). After 9 years, they found that SOC stocks in 0-10  
622 [cm had increased with addition rate, whereas SOC stock in 10-20 cm was similar in all](#)  
623 [treatments, indicating that the transport of SOC derived from maize residues was not](#)  
624 [detectable](#).

625

## 626 **5. Conclusion**

627 This study has demonstrated the consistency of a diachronic approach applied to one of the  
628 oldest LTE comparing contrasted tillage treatments. It allowed to make reliable conclusions on  
629 the effect of tillage on SOC storage: reduced tillage resulted in SOC storage in the upper soil  
630 layers (~0-10 cm) but simultaneously in a SOC decline in the underlying layers (~15-40 cm)  
631 and no change in SOC storage compared to conventional tillage over the whole sampling depth  
632 (~0-40 cm or ~0-60 cm). It confirms the importance of deep sampling and calculation at  
633 equivalent soil mass for comparing tillage treatments and avoiding misinterpretations (Powlson  
634 *et al.*, 2014). The natural <sup>13</sup>C tracing technique applied *in situ* showed that the C mineralization  
635 rate and the MRT in each soil layer varied with depth and tillage by a factor of 2.5. This  
636 amplitude of variation confirmed the results obtained in previous laboratory studies made on  
637 the same experiment. The C mineralization rate appeared to be controlled mainly by the  
638 intensity of the C inputs and not by the physical disturbance linked with tillage. Its variation is  
639 likely to be caused by the variation in priming effect with C addition rate. Incorporating this  
640 effect into the AMG model allowed to improve the simulation of SOC evolution in each soil  
641 layer and in the whole (old) ploughed layer without considering any other effect of tillage.  
642 Further investigations are required to evaluate the new model AMGv3 on other LTEs

643 comparing treatments with large variation in C inputs, ranging from bare fallow soils to cropping  
644 systems with intensive C inputs and grasslands.

645

646

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655

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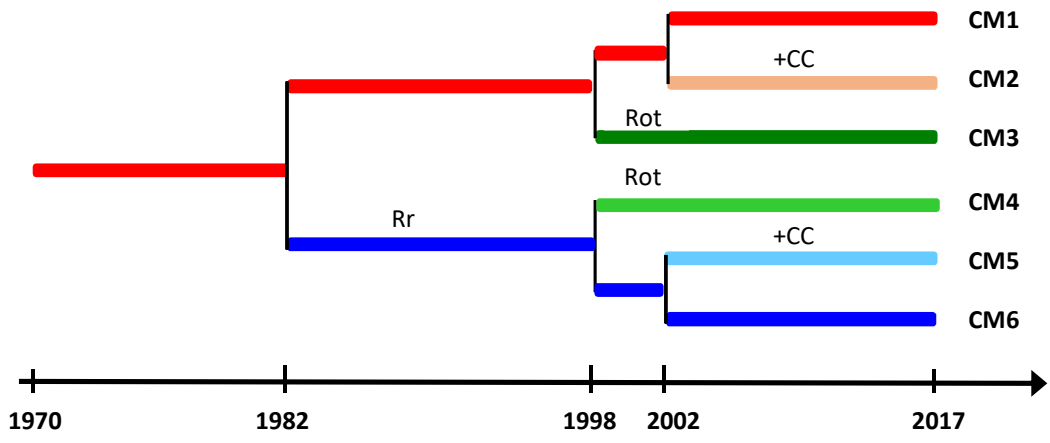
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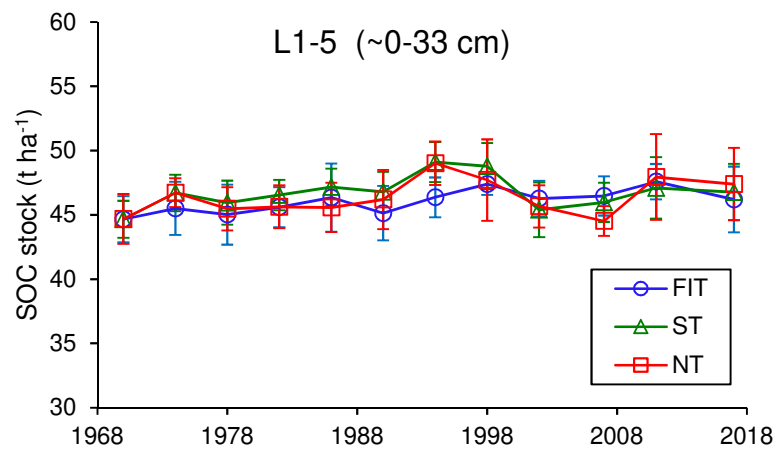
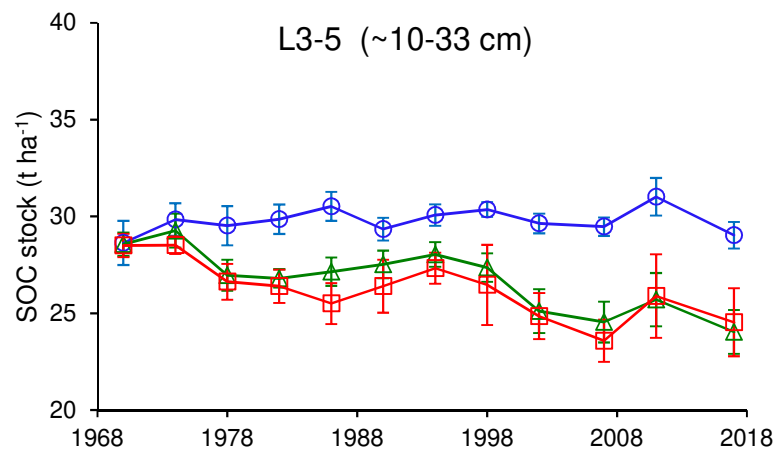
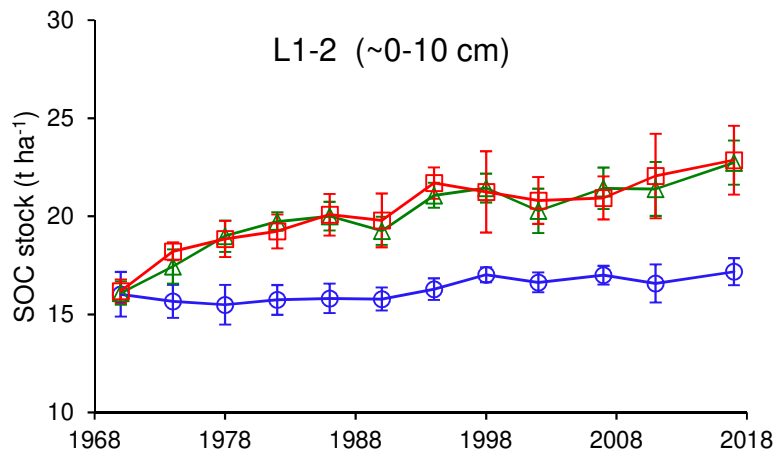
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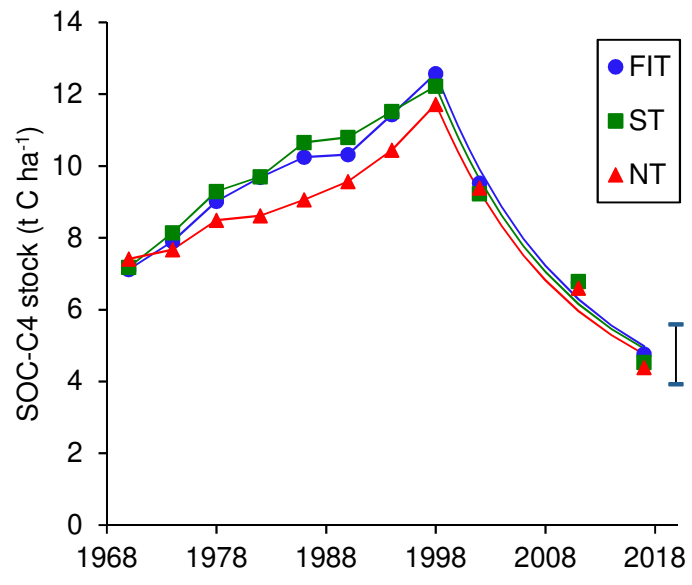
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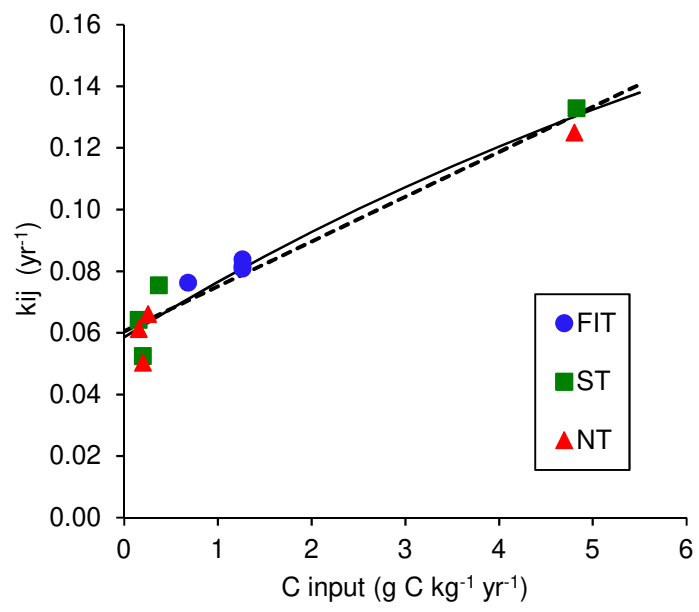
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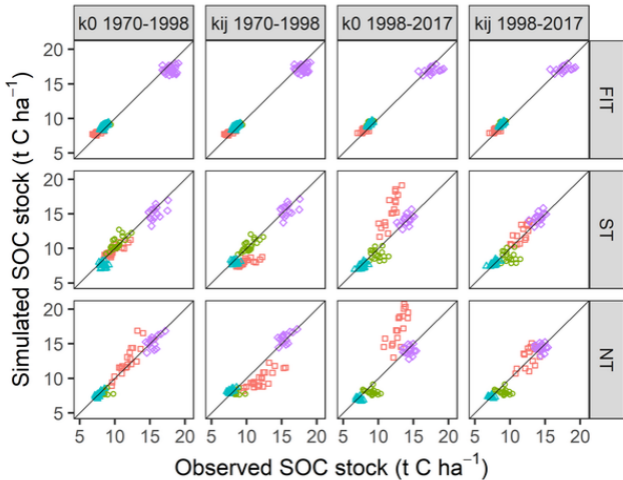
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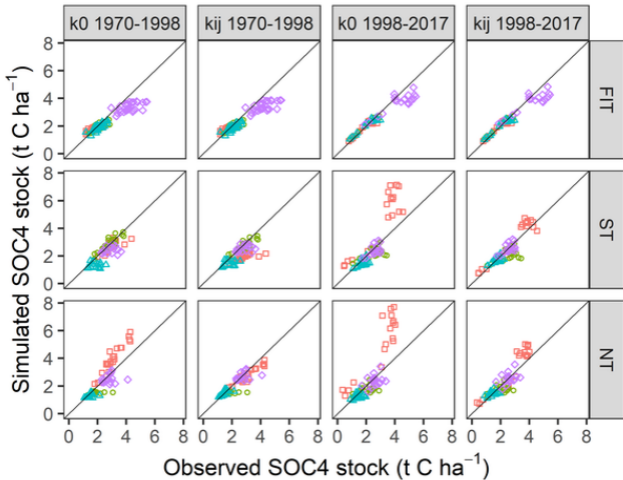








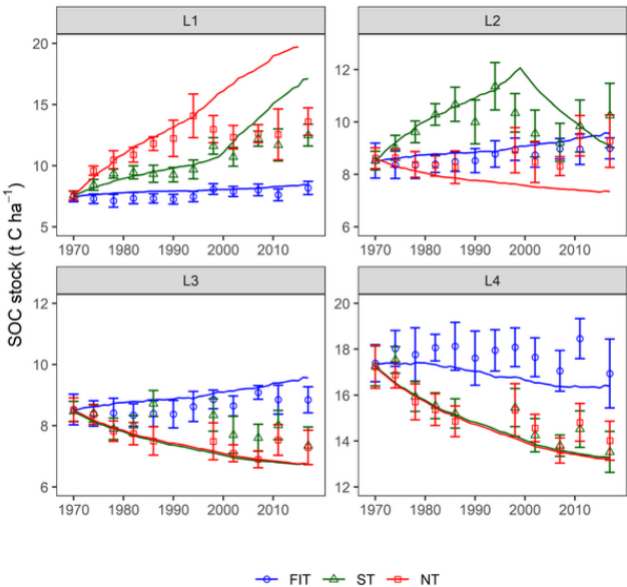
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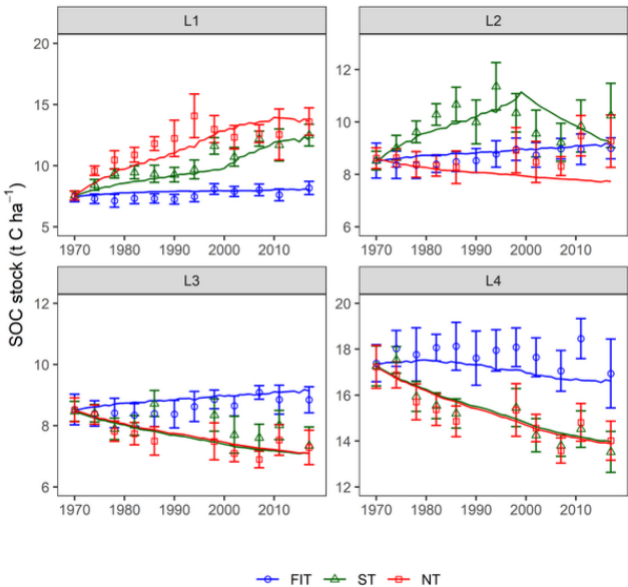
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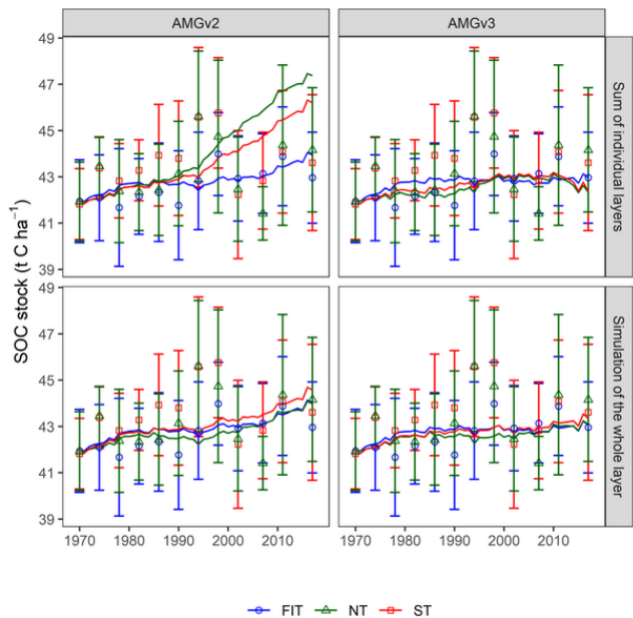


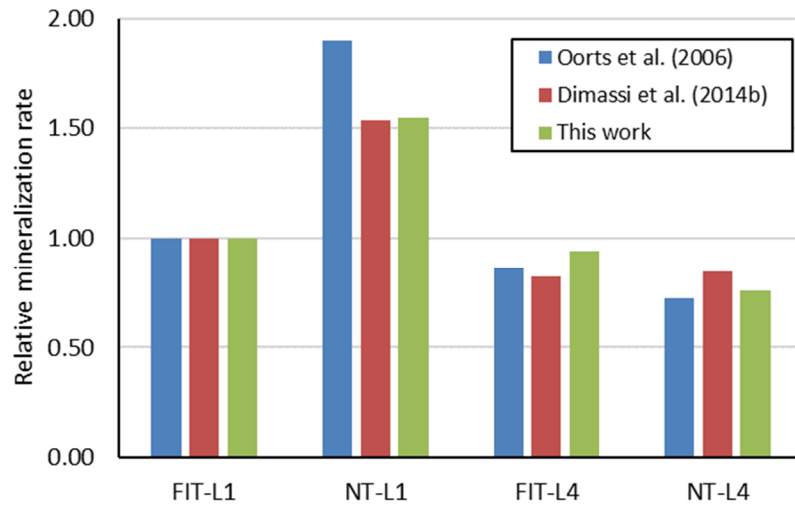
# a) AMGv2



## b) AMGv3







**Table 1.** SOC concentration and cumulative SOC stock per soil layer and the three tillage treatments in 2017 (mean of the six crop management treatments). Values in parentheses are the standard deviations. Letters indicate significant differences between tillage treatments ( $p < 0.05$ ).

Layer	Soil mass (t ha <sup>-1</sup> )	Depth (cm)	C concentration (g kg <sup>-1</sup> )			Layer	Soil mass (t ha <sup>-1</sup> )	Depth (cm)	Cumulative SOC stock (t C ha <sup>-1</sup> )		
			FIT	ST	NT				FIT	ST	NT
L1	700	~ 0-5	<b>11.78</b> (0.91) c	<b>18.04</b> (1.25) b	<b>19.67</b> (1.68) a	L1	700	~ 0-5	<b>8.18</b> (0.64) c	<b>12.49</b> (0.89) b	<b>13.60</b> (1.13) a
L2	800	~ 5-10	<b>11.28</b> (0.71) b	<b>12.80</b> (1.53) a	<b>11.59</b> (1.27) b	L1-2	1500	~ 0-10	<b>17.18</b> (1.11) b	<b>22.74</b> (1.70) a	<b>22.87</b> (1.74) a
L3	800	~ 10-15	<b>11.07</b> (0.67) a	<b>9.19</b> (0.75) b	<b>9.10</b> (0.67) b	L1-3	2300	~ 0-15	<b>26.02</b> (1.45) b	<b>30.08</b> (2.24) a	<b>30.15</b> (2.09) a
L4	1760	~ 15-28	<b>9.61</b> (1.02) a	<b>7.68</b> (0.52) b	<b>7.96</b> (0.49) b	L1-4	4060	~ 0-28	<b>42.96</b> (2.53) a	<b>43.61</b> (2.93) a	<b>44.17</b> (2.68) a
L5	540	~ 28-33	<b>6.03</b> (1.06) a	<b>5.83</b> (0.56) a	<b>5.96</b> (0.38) a	L1-5	4600	~ 0-33	<b>46.20</b> (2.84) a	<b>46.76</b> (3.08) a	<b>47.40</b> (2.75) a
L6	4000	~ 33-60	<b>4.75</b> (0.69) a	<b>4.81</b> (0.58) a	<b>4.89</b> (0.52) a	L1-6	8600	~ 0-60	<b>65.21</b> (4.96) a	<b>65.99</b> (4.27) a	<b>66.93</b> (3.66) a

**Table 2.** Quality of fit (RRMSE = relative RMSE) and mineralization rate constants ( $k_{ij}$ ) calculated during the decay of SOC-C4 stocks in treatments CM3 and CM4, per soil layer and tillage treatment. Values in parentheses are the standard deviations. Uppercase letters indicate significant differences between layers ( $p < 0.05$ ). Lowercase letters indicate significant differences between tillage treatments ( $p < 0.05$ ).

Layer	Depth (cm)	Tillage treatment					
		FIT		ST		NT	
		RRMSE	$k_{ij}$ ( $yr^{-1}$ )	RRMSE	$k_{ij}$ ( $yr^{-1}$ )	RRMSE	$k_{ij}$ ( $yr^{-1}$ )
<b>L1</b>	~ 0-5	0.054	<b>0.081</b> (0.018) A b	0.168	<b>0.133</b> (0.025) A a	0.213	<b>0.125</b> (0.028) A a
<b>L2</b>	~ 5-10	0.055	<b>0.082</b> (0.016) A a	0.067	<b>0.075</b> (0.013) B a	0.117	<b>0.066</b> (0.015) B a
<b>L3</b>	~ 10-15	0.056	<b>0.084</b> (0.019) A a	0.105	<b>0.053</b> (0.017) C b	0.115	<b>0.050</b> (0.004) B b
<b>L4</b>	~ 15-28	0.072	<b>0.076</b> (0.016) A a	0.126	<b>0.064</b> (0.030) BC a	0.120	<b>0.061</b> (0.011) B a
<b>L1-4</b>	~ 0-28	0.059	<b>0.080</b> (0.017) a	0.068	<b>0.079</b> (0.019) a	0.113	<b>0.080</b> (0.010) a

**Table 3.** C inputs per soil layer: mean values (t C ha<sup>-1</sup> yr<sup>-1</sup>) calculated over the periods 1970-1998 and 1998-2017. Values in parenthesis (sd) are the standard deviations between crop management treatments. Calculations are based on the method of Bolinder *et al.* (2003) with non allometric root:shoot ratio, as described by Clivot *et al.* (2019).

Layer	Depth (cm)	C inputs (t C ha <sup>-1</sup> yr <sup>-1</sup> )					
		FIT		ST		NT	
		mean	sd	mean	sd	mean	sd
a) Period 1970-1998							
L1	~ 0-5	0.61	(0.10)	1.20	(0.39)	2.33	(0.37)
L2	~ 5-10	0.70	(0.11)	1.38	(0.08)	0.22	(0.03)
L3	~ 10-15	0.70	(0.11)	0.17	(0.02)	0.17	(0.02)
L4	~ 15-28	1.06	(0.14)	0.31	(0.04)	0.31	(0.04)
<b>L1-4</b>	<b>~ 0-28</b>	<b>3.06</b>	<b>(0.45)</b>	<b>3.06</b>	<b>(0.52)</b>	<b>3.03</b>	<b>(0.46)</b>
L1-5	~ 0-33	3.13	(0.46)	3.12	(0.53)	3.09	(0.47)
b) Period 1998-2017							
L1	~ 0-5	0.88	(0.12)	3.38	(0.53)	3.36	(0.45)
L2	~ 5-10	1.01	(0.14)	0.30	(0.04)	0.20	(0.04)
L3	~ 10-15	1.01	(0.14)	0.16	(0.03)	0.16	(0.03)
L4	~ 15-28	1.20	(0.16)	0.27	(0.06)	0.27	(0.06)
<b>L1-4</b>	<b>~ 0-28</b>	<b>4.10</b>	<b>(0.56)</b>	<b>4.11</b>	<b>(0.67)</b>	<b>4.00</b>	<b>(0.59)</b>
L1-5	~ 0-33	4.16	(0.58)	4.16	(0.68)	4.05	(0.60)

**Table 4.** Statistical evaluation of AMG model for predicting SOC and SOC4 stocks in all situations (6 crop managements x 11 dates) for each layer (L1-L4) and each tillage system (FIT, NT or NT). MD = mean difference (t C ha<sup>-1</sup>); RMSE = root mean square error (t C ha<sup>-1</sup>).

Mineralization rate model *	Layer	Depth cm	SOC						SOC4					
			MD			RMSE			MD			RMSE		
			FIT	ST	NT	FIT	ST	NT	FIT	ST	NT	FIT	ST	NT
<i>k<sub>0</sub></i>	L1	~ 0-5	0.42	1.32	2.94	0.54	2.41	4.15	-0.03	0.44	1.81	0.25	1.07	2.21
	L2	~ 5-10	0.37	0.29	-1.11	0.45	1.04	1.41	-0.19	-0.01	-0.52	0.30	0.39	0.66
	L3	~ 10-15	0.45	-0.78	-0.23	0.51	0.93	0.45	-0.18	-0.62	-0.28	0.28	0.70	0.38
	L4	~ 15-28	-0.99	-0.57	-0.59	1.31	0.99	0.99	-1.01	-0.61	-0.53	1.15	0.73	0.70
	L1-4 **	~ 0-28	0.25	0.01	1.09	1.16	2.40	2.81	-1.42	-0.86	0.47	1.80	1.29	1.19
<i>k<sub>ij</sub></i>	L1	~ 0-5	0.42	-1.23	-1.30	0.54	1.60	1.93	-0.03	-0.60	0.06	0.25	0.84	0.56
	L2	~ 5-10	0.34	-0.24	-1.09	0.44	1.03	1.40	-0.20	-0.23	-0.52	0.31	0.48	0.65
	L3	~ 10-15	0.37	-0.25	0.41	0.44	0.52	0.53	-0.21	-0.41	-0.04	0.30	0.51	0.22
	L4	~ 15-28	-0.76	-0.12	0.06	1.13	0.83	0.75	-0.92	-0.44	-0.28	1.06	0.58	0.50
	L1-4 **	~ 0-28	0.38	-2.08	-1.83	1.18	2.83	2.78	-1.37	-1.75	-0.78	1.76	2.01	1.27
<i>k<sub>0</sub>(I)</i>	L1	~ 0-5	0.30	-0.50	-0.40	0.48	0.86	1.40	-0.09	-0.30	0.44	0.30	0.53	0.67
	L2	~ 5-10	0.23	-0.16	-0.78	0.39	0.88	1.13	-0.25	-0.20	-0.39	0.37	0.43	0.54
	L3	~ 10-15	0.31	-0.46	0.09	0.40	0.66	0.37	-0.24	-0.50	-0.16	0.34	0.59	0.29
	L4	~ 15-28	-0.76	0.08	0.05	1.15	0.83	0.74	-0.92	-0.37	-0.29	1.07	0.52	0.51
	L1-4 **	~ 0-28	0.08	-1.29	-0.95	1.32	2.36	2.25	-1.50	-1.43	-0.41	1.94	1.75	1.05

\* *k<sub>0</sub>* = standard mineralization rate (model AMGv2); *k<sub>ij</sub>* = fixed mineralization rates per layer and treatment (Table 2); *k<sub>0</sub>(I)* = mineralization rate varying with C input (Eq. 5) (model AMGv3).

\*\* Sum of individual simulations of layers L1, L2, L3 and L4.



