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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**

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23 **Keywords:** soil organic carbon, SOC, ¹³C, mineralization rate, tillage, carbon inputs, priming,
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 ¹³C 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 ¹³C 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⁻¹
152 yr⁻¹.

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 ¹³C 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 CaCO₃ 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 CaCO₃ kg⁻¹ 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 ¹³C 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 ¹³C 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⁻¹
198 corresponding to ~ 0-5 cm); L2 (800 t ha⁻¹, ~ 5-10 cm); L3 (800 t ha⁻¹, ~ 10-15 cm); L4 (1760 t
199 ha⁻¹, ~ 15-28 cm); L5 (540 t ha⁻¹, ~ 28-33 cm) and L6 (4000 t ha⁻¹, ~ 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⁻¹, 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⁻¹) 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⁻³) and the SOC concentration (g
213 kg⁻¹) 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
$$SOC4 = f \cdot SOC \quad (2)$$

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

220 where f is the proportion of SOC derived from C4 plants, δ is the $\delta^{13}C$ signature of the SOC, δ_3
221 and δ_4 are the $\delta^{13}C$ signatures of the C inputs derived from C3 plants and C4 plants
222 respectively. The values of δ_3 and δ_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
$$SOC4 = (C_{04} - C_{s4}) \cdot \exp(-k \cdot t) + C_{s4} \quad (4)$$

231 where C_{04} is the initial SOC4 stock (in 1998), k is the mineralization rate (expressed per unit
232 of SOC, in 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 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 CaCO₃ contents were considered invariant and homogeneous between
 296 layers and treatments (239 and 0 g kg⁻¹ 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 SOC₄. 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 SOC₄ 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 α represents the mineralization rate in the absence of C input, β 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 ± 4.9 , 66.0 ± 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 ± 0.8 and 5.5 ± 0.9 t C ha⁻¹ in layer L1-2 and decreased by -4.9 ± 0.7
350 and -4.4 ± 0.8 t C ha⁻¹ 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⁻¹ respectively) than in the FIT treatment (0.081 yr⁻¹). 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 ± 0.001 yr⁻¹) 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 ± 0.03
383 yr⁻¹.

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 ± 0.02 to 4.06 ± 0.06 t C ha⁻¹ yr⁻¹ 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⁻¹ 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⁻¹), as expected.
402 We also found a close correlation between k_{ij} and the mean CPOM content (P , expressed in
403 g C kg⁻¹ 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⁻¹ with the
423 standard mineralization rate to 1.22 t C ha⁻¹ 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⁻¹ with the k_0
437 mineralization rate and only 1.93 t C ha⁻¹ 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⁻¹ with k_0 to 0.56 t
440 C ha⁻¹ 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 α , β 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⁻¹ soil yr⁻¹, 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⁻¹ yr⁻¹), should have
451 almost doubled the potential mineralization rate, from 0.31 to 0.55 yr⁻¹. Conversely, the
452 reduction in C input from 1.0 to 0.2 g C kg⁻¹ soil yr⁻¹, 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⁻¹.

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⁻¹). The quality
459 of fit was better in the new model $k_0(I)$ compared to the k_{ij} 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⁻¹
461 respectively, and the mean **absolute MD** was 0.34, 0.55 and 0.84 t C ha⁻¹. 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 ± 0.03 and -0.09 ± 0.04 t C ha⁻¹
494 yr⁻¹ (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 ¹³C 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 ± 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}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 ¹³C 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 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 ¹³C 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 ¹³C 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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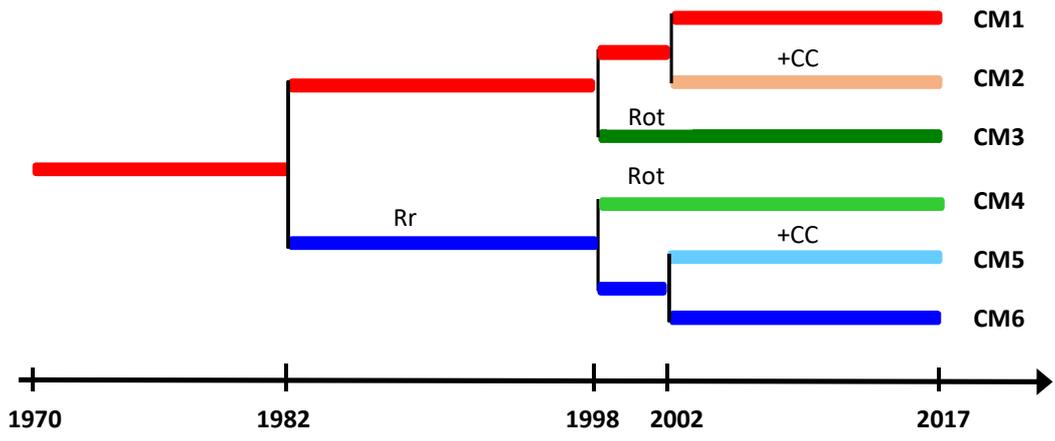
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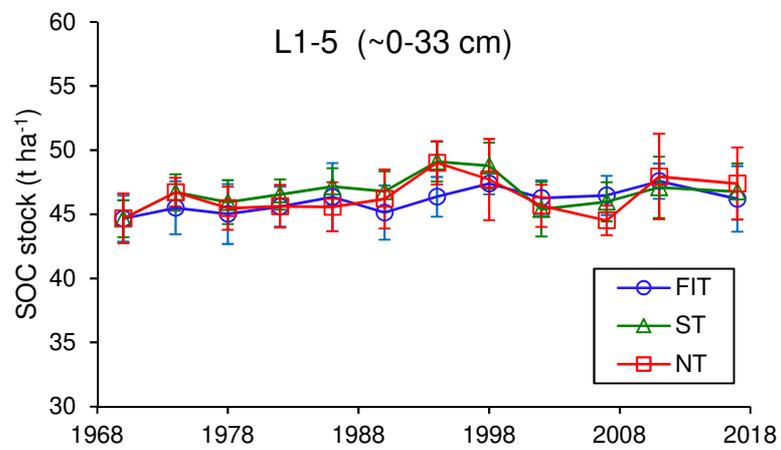
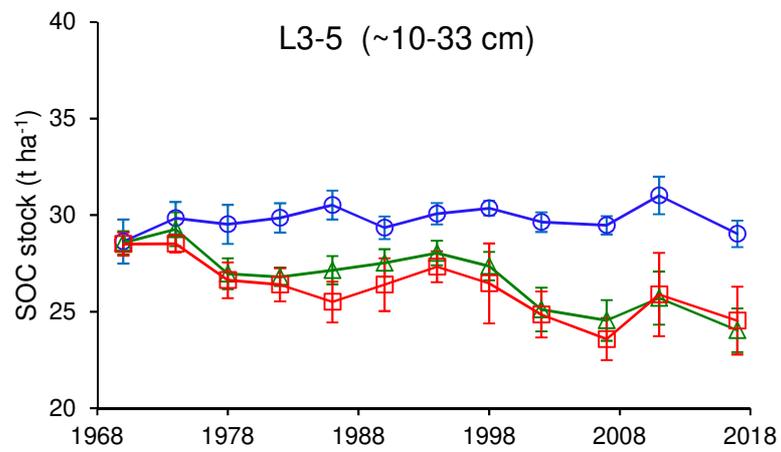
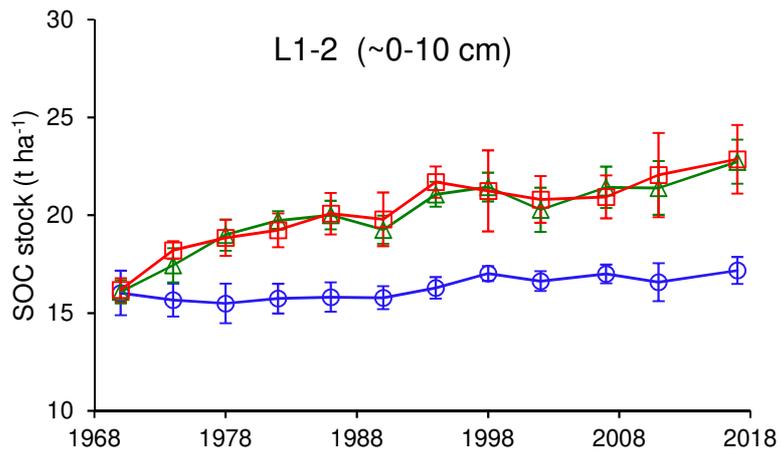
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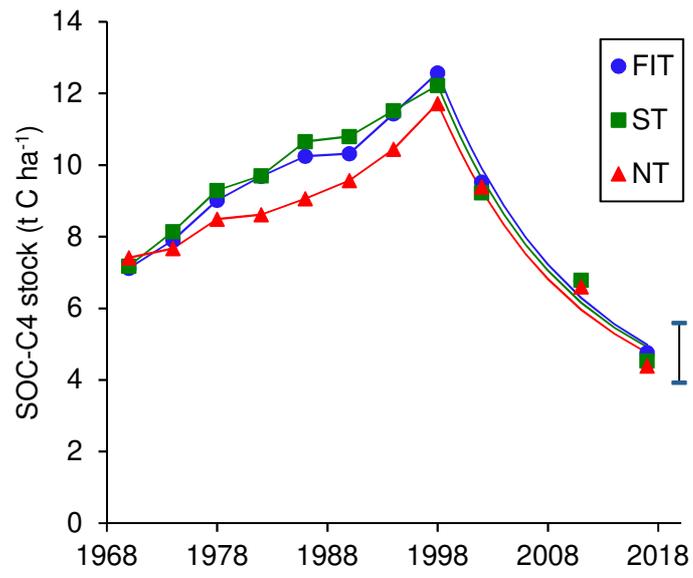
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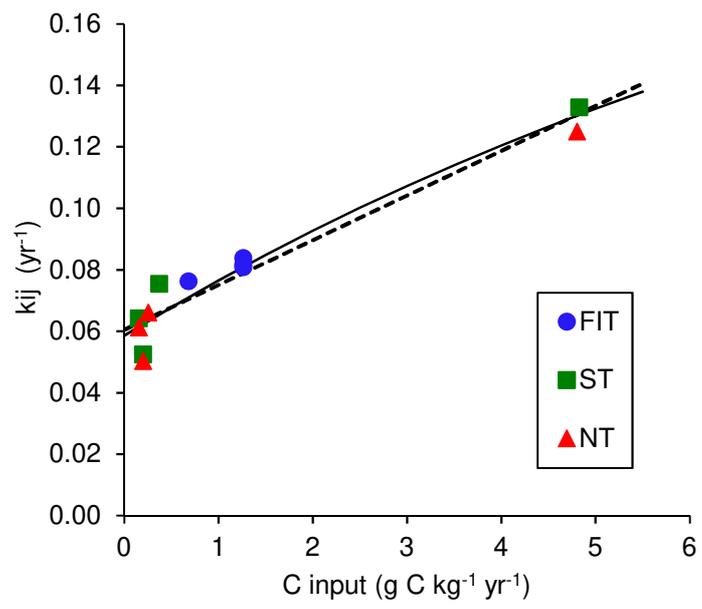
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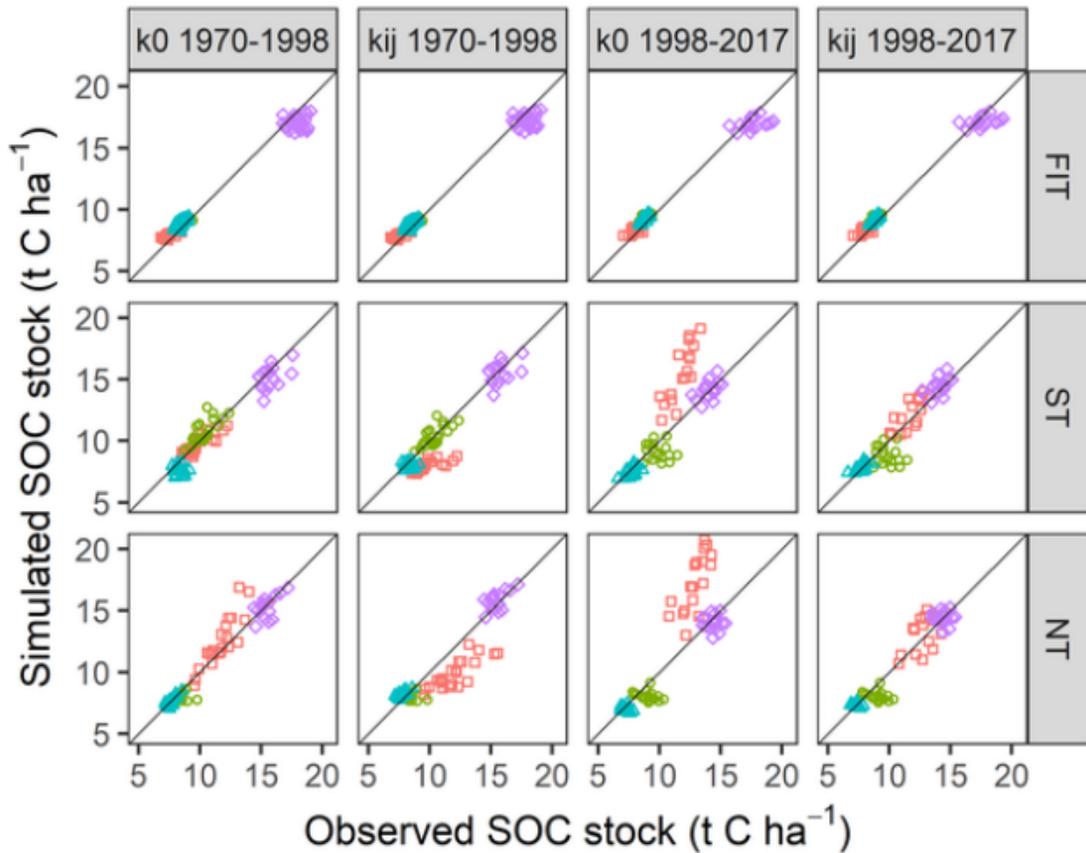
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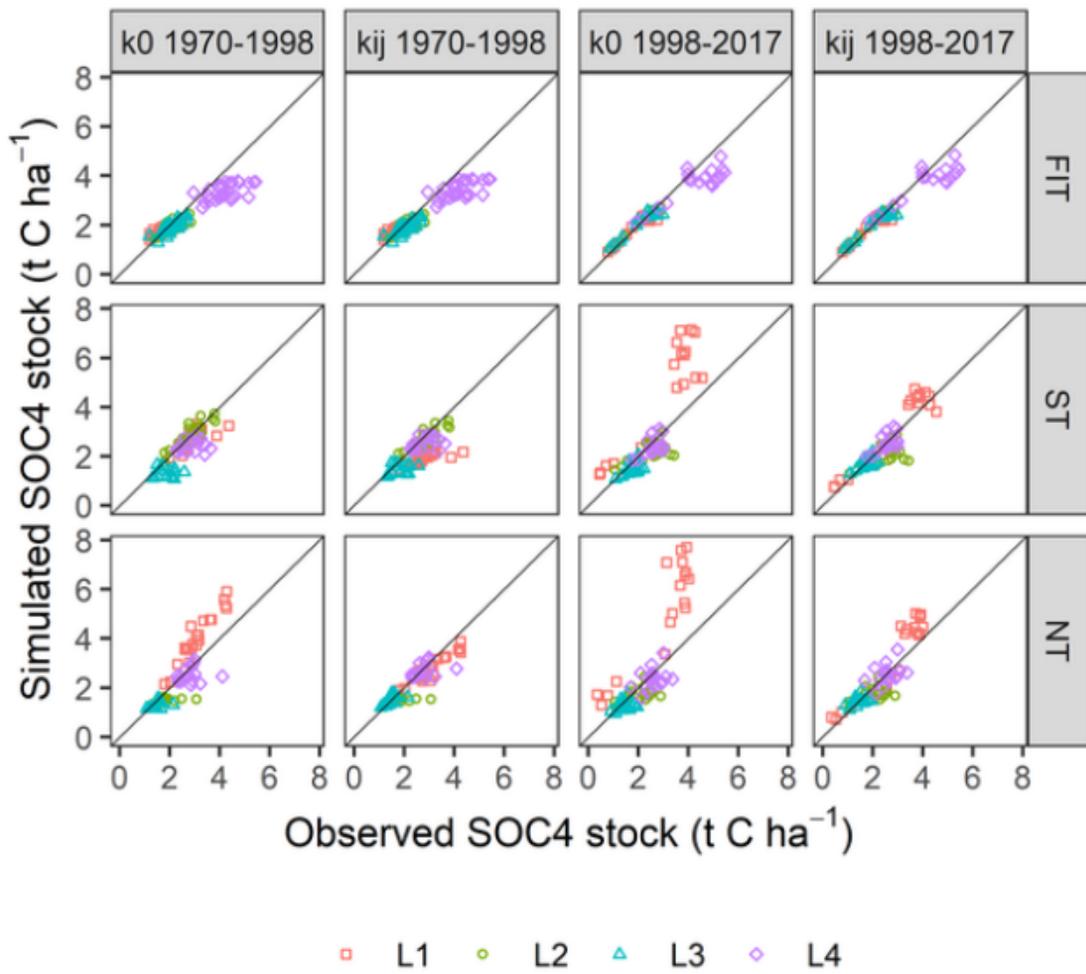




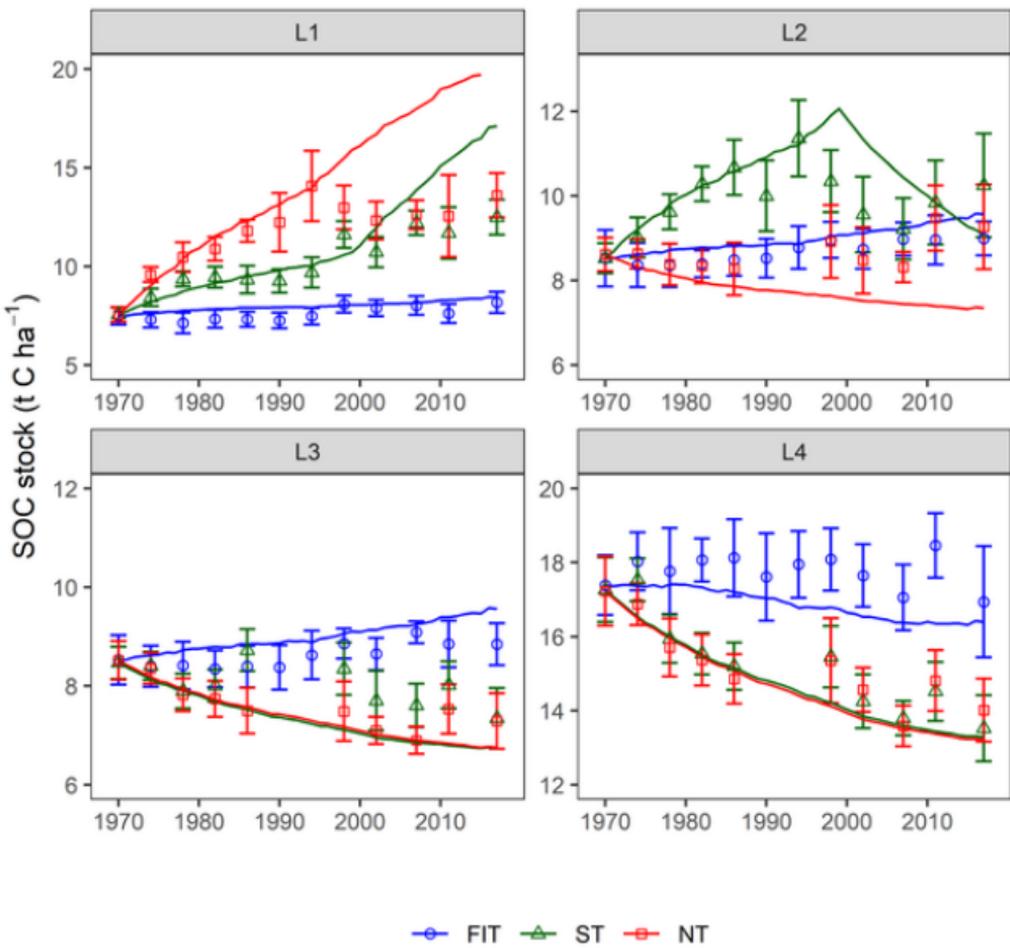
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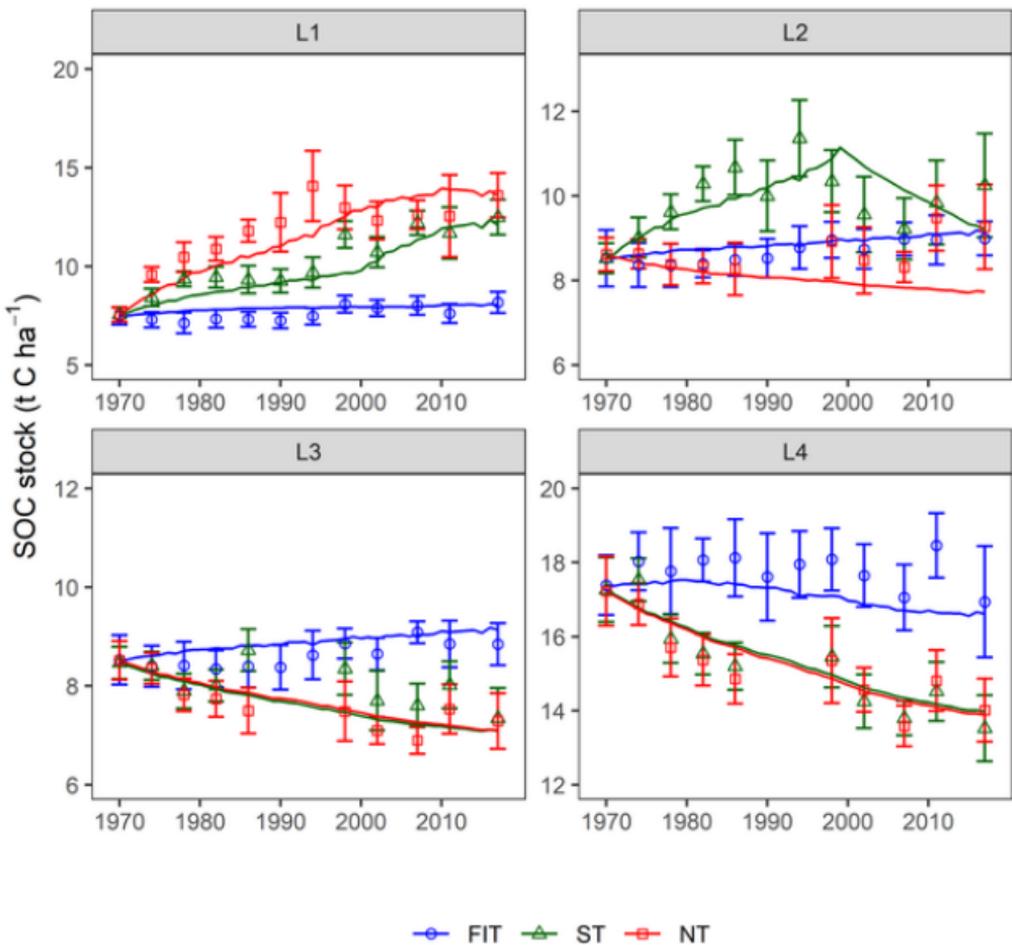
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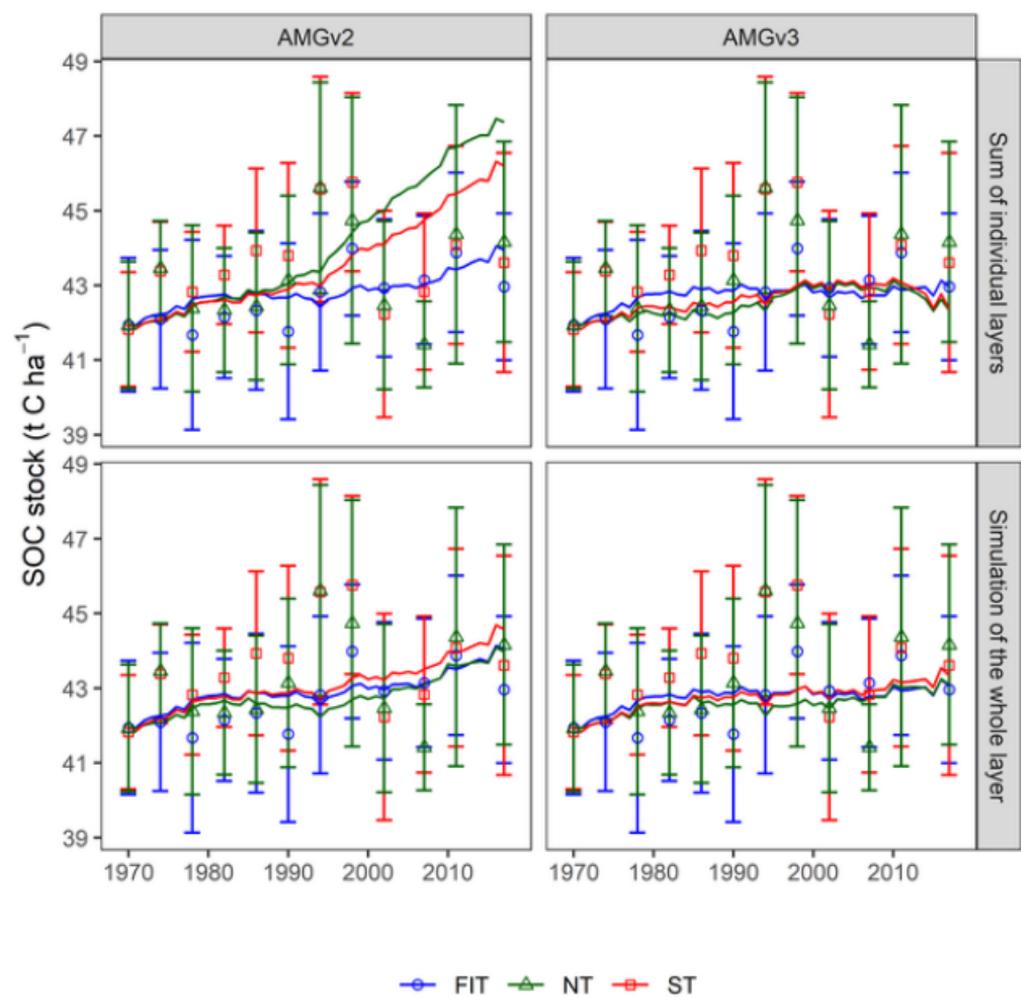


a) AMGv2



b) AMGv3





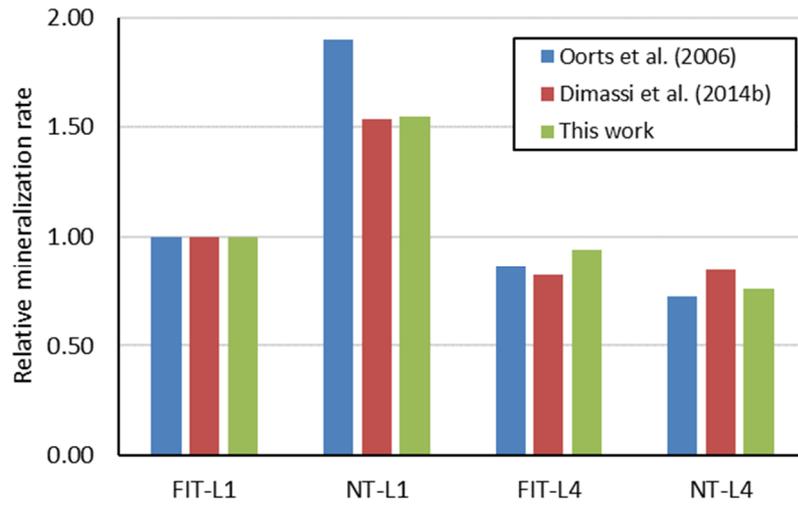


Table 1. SOC concentration and cumulative SOC stock per soil layer and the three tillage treatments in 2017 (mean of the six crop management treatments). Values in parentheses are the standard deviations. Letters indicate significant differences between tillage treatments ($p < 0.05$).

Layer	Soil mass (t ha ⁻¹)	Depth (cm)	C concentration (g kg ⁻¹)			Layer	Soil mass (t ha ⁻¹)	Depth (cm)	Cumulative SOC stock (t C ha ⁻¹)		
			FIT	ST	NT				FIT	ST	NT
L1	700	~ 0-5	11.78 (0.91) c	18.04 (1.25) b	19.67 (1.68) a	L1	700	~ 0-5	8.18 (0.64) c	12.49 (0.89) b	13.60 (1.13) a
L2	800	~ 5-10	11.28 (0.71) b	12.80 (1.53) a	11.59 (1.27) b	L1-2	1500	~ 0-10	17.18 (1.11) b	22.74 (1.70) a	22.87 (1.74) a
L3	800	~ 10-15	11.07 (0.67) a	9.19 (0.75) b	9.10 (0.67) b	L1-3	2300	~ 0-15	26.02 (1.45) b	30.08 (2.24) a	30.15 (2.09) a
L4	1760	~ 15-28	9.61 (1.02) a	7.68 (0.52) b	7.96 (0.49) b	L1-4	4060	~ 0-28	42.96 (2.53) a	43.61 (2.93) a	44.17 (2.68) a
L5	540	~ 28-33	6.03 (1.06) a	5.83 (0.56) a	5.96 (0.38) a	L1-5	4600	~ 0-33	46.20 (2.84) a	46.76 (3.08) a	47.40 (2.75) a
L6	4000	~ 33-60	4.75 (0.69) a	4.81 (0.58) a	4.89 (0.52) a	L1-6	8600	~ 0-60	65.21 (4.96) a	65.99 (4.27) a	66.93 (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})
L1	~ 0-5	0.054	0.081 (0.018) A b	0.168	0.133 (0.025) A a	0.213	0.125 (0.028) A a
L2	~ 5-10	0.055	0.082 (0.016) A a	0.067	0.075 (0.013) B a	0.117	0.066 (0.015) B a
L3	~ 10-15	0.056	0.084 (0.019) A a	0.105	0.053 (0.017) C b	0.115	0.050 (0.004) B b
L4	~ 15-28	0.072	0.076 (0.016) A a	0.126	0.064 (0.030) BC a	0.120	0.061 (0.011) B a
L1-4	~ 0-28	0.059	0.080 (0.017) a	0.068	0.079 (0.019) a	0.113	0.080 (0.010) a

Table 3. C inputs per soil layer: mean values (t C ha⁻¹ yr⁻¹) 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 ⁻¹ yr ⁻¹)					
		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)	
L1-4	~ 0-28	3.06 (0.45)		3.06 (0.52)		3.03 (0.46)	
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)	
L1-4	~ 0-28	4.10 (0.56)		4.11 (0.67)		4.00 (0.59)	
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⁻¹); RMSE = root mean square error (t C ha⁻¹).

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>k₀</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_{ij}</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>k₀(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

* *k₀* = standard mineralization rate (model AMGv2); *k_{ij}* = fixed mineralization rates per layer and treatment (Table 2); *k₀(I)* = mineralization rate varying with C input (Eq. 5) (model AMGv3).

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

