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Similar specific mineralization rates of organic carbon and nitrogen in incubated soils under contrasted arable cropping systems

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Highlights:

- C and N mineralization were compared in four arable cropping systems after 17 years
- Incubations were realized on disturbed or undisturbed soil cores
- C and N mineralized were greater in undisturbed than in disturbed soil samples
- Specific C and N mineralization rates did not differ among the four cropping systems

Abstract

No tillage is often thought to mitigate greenhouse gas emissions from agricultural land by increasing soil carbon storage, because of a reduced mineralization of soil organic carbon (SOC) and nitrogen (SON). Regrettably, most available references on this topic come from laboratory incubations of disrupted soil from superficial soil layer. Here, we compare SOC and SON mineralization rates in the long-term experiment of La Cage (France) under conventional (CON), low input (LI), conservation agriculture (CA) or organic (ORG) management. Disturbed soil samples from the 0-27 cm soil layer of all treatments were laboratory incubated for four months, while undisturbed CON and CA soil cores were incubated to account for tillage effects. Physical disturbance decreased SOC and SON mineralization. Model fitting showed that the size of the C labile pool and the C and N mineralization rates of the slow pool were 1.5 to 2.3-fold greater in undisturbed soil cores than in disturbed ones, which may be due to a higher abundance of labile SOC (*e.g.* plant residues) in undisturbed soil cores. All cropping systems exhibited similar specific rate of mineralization, expressed per unit of SOC, SON or microbial biomass C, both for disturbed and undisturbed soils. Similar mineralization in CA and CON undisturbed soil cores may result from the balance between higher amount of labile OM and less favourable soil structure for decomposition in CA. Similar mineralization rates in disturbed soil cores suggest that OM decomposability and environmental conditions for decomposers were similar between cropping systems. Overall, these results confirmed the hypothesis previously made *in silico* to explain the differences in SOC storage in this experiment (Autret *et al.*, 2016). Our results together with the increased SOC stocks observed in CA and ORG treatments suggest that increased biomass returns to soil or changes in microbial physiology may be the main drivers of SOC storage.

1. Introduction

Agricultural practices can play a major role in mitigating GHG emissions of agriculture through decreased GHG emissions from soils and increased soil organic carbon (SOC) stocks (Smith *et al.*, 2008). A range of agricultural practices have been identified to increase SOC stocks, such as the choice of the rotation, residue return, cover crops, agroforestry and reduced tillage (Smith *et al.*, 2008; Stockmann *et al.*, 2013; Paustian *et al.*, 2016). While extensively reviewed at the global scale (see previous references) or at the national scale (*e.g.* VandenBygaart *et al.*, 2008; Pellerin *et al.*, 2013), there is still a large uncertainty on the potential SOC storage rate of the different practices. The effect of some of these practices has been re-evaluated with smaller SOC storage rates than previously thought, particularly for tillage (Luo *et al.*, 2010; Virto *et al.*, 2012; Meurer *et al.*, 2018). Alternative cropping systems that have been identified to provide environmental benefits associate various practices. Compared to conventional systems, low input systems, also called integrated systems, combine a reduction in N fertilization, pesticide application and tillage intensity. Organic agriculture, which excludes synthetic fertilizers and pesticides, promotes the use of organic fertilizers, legumes and green manures to provide nutrients to crops and more frequent tillage to control weeds. Conservation agriculture combines the absence of tillage with permanent plant cover and more diversified rotations. Most of the literature dealing with SOC storage in soils concerns agricultural practices rather than cropping systems.

Cropping systems and agricultural practices may increase SOC stocks by either increasing OC (organic C) inputs to soil or decreasing SOC mineralization rate. In fact, very few studies allow to compare the contribution of these two levers, increasing OC inputs and decreasing SOC outputs, to changes in SOC stocks with management. Two recent studies suggest that increased SOC storage is rather due to increased OC inputs than to reduction in mineralization rates of SOC. In an alley cropping agroforestry system, Cardinael *et al.* (2018) found that biomass-C inputs to soil were increased compared to a reference plot thanks to the tree rows. They demonstrated in a modelling exercise that these inputs could explain the observed increase in SOC stock in the agroforestry plot. Another study analyzed the evolution of SOC stocks during 16 years in alternative cropping systems, *i.e.* low input (LI), organic (ORG), conservation agriculture (CA), compared to a conventional system (CON) (Autret *et al.*, 2016). It showed that SOC stocks increased markedly in the CA and to a lesser extent in the ORG system, but not in the other two systems. OC inputs to soil, assessed over the 16 years, were increased in the CA compared to the CON system. The model AMG (Saffih-Hdadi & Mary, 2008) was able to reproduce the evolution of SOC stocks in the four systems using a single decomposition rate constant. This suggests that specific mineralization rates (per unit of SOC) may be independent of the cropping system, and may be similar in systems with or without tillage.

This result is contrary to the current paradigm relative to the effect of no tillage. Compared to conventional tillage with full inversion, no tillage is supposed to decrease SOC mineralization rates because of less favourable local climatic conditions and better physical protection of organic matter in soil aggregates (Balesdent *et al.*, 2000). Several studies, using ^{13}C natural abundance methodology, indicated that the mean residence time of SOC under no tillage was 2.1 (Balesdent *et al.*, 1990), 1.9 (Ryan *et al.*, 1995) and 1.7 times higher (Six *et al.*, 1998) under no tillage than under full inversion tillage for the equivalent of the ploughed layer (Paustian *et al.*, 2000). However, using the same technique, Haile-Mariam *et al.* (2008) did not find any difference in MRT between no-till and tilled systems in three other long term experiments in USA. In other studies which simulated the dynamics of SOC in long term experiments, the decay rate constants of SOC under no tillage were found to be 0.73 (Huggins *et al.*, 2007), 0.48 (Chatskikh *et al.*, 2009) and 0.88 (Dimassi, 2013) relative to full inversion tillage, also for the equivalent of the ploughed layer. Another way to estimate the decomposition rate of SOC is to measure mineralization rates in incubation experiments. However, published results are highly variable and often based on very superficial layers of soil (*e.g.* 0-5 or 0-10 cm), biasing the analysis since these layers are relatively enriched in SOC and easily decomposable organic matter under no till. Incubation experiments often use disturbed soil samples, in which the initial soil structure is disrupted and soil samples are sieved. This disruption was suspected to increase the mineralization rates of SOC and SON (Balesdent *et al.*, 2000) also providing an explanation for the effects of tillage on mineralization of soil organic matter (Beare *et al.*, 1994), *i.e.* to protect soil organic matter from decomposition in aggregates not frequently disturbed by tillage operations nor exposure to rain at the soil surface (Balesdent *et al.*, 2000).

The aim of this paper was to test the conclusion of Autret *et al.* (2016) that specific mineralization rates of SOC were unaffected under alternative cropping systems in a temperate Luvisol. We hypothesized that specific mineralization rates (per unit of SOC or SON) would be the same in soil samples from the different cropping systems. To test this, we sampled soil in the ploughed or equivalent layer (0-27 cm) in plots under the four cropping systems, incubated the soil in the laboratory and measured the net C and N mineralization over time. We tested the effect of physical disturbance by comparing incubation of disturbed and sieved soils to incubation of intact soil cores, hypothesizing that mineralization rates would be higher in disrupted soils, but also anticipating the presence of coarse fresh organic matter in the cores.

2. Materials and methods

2.1 Site and soil characteristics

We investigated soil samples from the long-term field experiment of “La Cage” located in Versailles, France (48°48’ N, 2°08’ E). The experiment was started in 1998 and aimed to evaluate the agronomic, economic and environmental performances of low input (LI), conservation agriculture (CA) (direct seeding under permanent plant cover) and organic farming (ORG) systems compared to conventional farming (CON) (Balabane *et al.*, 2005). Long term annual mean temperature is 11.3 °C and annual rainfall averages 627 mm. The soil is a well-drained deep Luvisol (IUSS Working Group WRB, 2006), with a particle size distribution of 17 % clay, 56 % silt and 27 % sand. At the start of the experiment, the ploughed layer (0-30 cm) had a C/N ratio of 9.6, a pH of 7.38 and a mean organic C content of 9.49 g kg^{-1}. In 2014, its mean SOC content was 9.70, 10.21, 9.72 and 12.07 g kg^{-1} in CON, LI, ORG and CA respectively (Autret *et al.*, 2016).

2.2.Cropping systems

The field experiment is arranged in a randomized complete block design, divided into two blocks, themselves divided into four plots for each cropping system. Each plot is divided into two subplots of 0.56 ha, so that two different crops of the crop rotation are present each year, wheat being grown every year in one of the two subplots. A detailed presentation of crop rotations, soil management and fertilization was given by Autret *et al.* (2016). The 4 year crop rotation mainly consisted of rapeseed (*Brassica napus L.*), winter wheat (*Triticum aestivum L.*), spring pea (*Pisum sativum L.*) and winter wheat. It differed in CA and ORG for some years, with the replacement of rapeseed by maize (*Zea mays L.*) in CA or the introduction of alfalfa (*Medicago sativa*) in CA and ORG. The CA system includes a permanent soil cover, initially composed of fescue (*Festuca rubra*) and then alfalfa since 2008, grown under the main crops except pea. The rotation in the ORG system is alfalfa-alfalfa-wheat-wheat. The CON system is characterized by a soil and crop management representative of the Paris Basin cereal production, with annual soil ploughing, the absence of organic amendment, a mineral N fertilization (average rate = 143 kg N ha^{-1} yr^{-1}) and a systematic use of pesticides. Compared to CON, the LI system is managed with a less intensive soil tillage (one year out of two), a reduced mineral N fertilization (114 kg N ha^{-1} yr^{-1}) and a limited use of pesticides. The CA system had a reduced mineral N fertilization (104 kg N ha^{-1} yr^{-1}) as well, but the absence of soil tillage resulted in a systematic use of herbicides to destroy the cover crops and weeds. The ORG system was managed according to the European specifications for organic farming, *i.e.* without any application of synthetic N fertilizer or pesticides. More frequent soil tillage was practiced in ORG to control weeds growth.

2.3.Soil sampling and analysis

Soil sampling was performed in February 2016 in all plots of the experiment, between the rows of rapeseed and winter wheat. Two types of soil samples were collected:

- disturbed soil samples: 6 soil cores were taken randomly per plot in both blocks, down to 27 cm (current ploughing depth) using a cylindrical probe of 6 cm diameter. Soil cores taken in each plot were pooled and homogenized by mixing and sieving, and split into 16 subsamples of approximately 6 kg each. Coarse residues and visible roots were removed manually.
- undisturbed soil samples: 6 soil cores were taken randomly in each of the eight plots corresponding to the CON and CA systems, down to 27 cm depth using polyethylene (PE) tubes of 6 cm diameter. The 48 soil cores were kept intact (“undisturbed”) in their PE

tubes and visible crop residues located on the soil surface were removed manually. These undisturbed soil cores were sampled in order to evaluate the effect of no tillage (CA) on C and N mineralization compared to the conventionally ploughed system (CON).

The 64 soil samples (16 disturbed and 48 undisturbed) were kept in a cold room at 4°C during 2 weeks until the start of the incubation, in order to limit the process of mineralization.

2.4.Incubations

The details of the incubation conditions and the preparation of the soil samples are given in Figure 1. Soil samples were incubated at a temperature of 20°C and a soil moisture adjusted to 18.3% (g/g, equivalent to pF = 3), for a total incubation period of 4 months. A one-week pre-incubation was realized in order to reactivate the microbial flora and avoid any flush of C and N mineralization.

The 16 disturbed soil samples were prepared as follows. They were sieved through a 10 mm sieve. Soil moisture was determined and adjusted to obtain the required incubation moisture content if necessary. For C mineralization, three soil samples of 50 g each were placed in 0.5 L jars hermetically closed, with a small beaker of water to avoid soil drying. The follow-up of CO_2 emissions was carried out over 8 dates (day 1, 4, 7, 14, 29, 62, 91 and 118). Net N mineralization was determined on three replicated soil samples at three dates (day 0, 29 and 118), and on a single soil sample at four other dates (day 7, 14, 62 and 91), given the destructive nature of the measurement.

Undisturbed cores were prepared for incubation as follows. The soil cylinders were capped at the bottom with a cheesecloth cover, taped to the outer wall of the cylinder. Each cylinder was placed in a 2 L incubation jar on a 10 mm thick crosspiece in order to allow free gas diffusion through the base of the core. A 10 mL cup of water was placed in the incubation jar to avoid soil drying. Two beakers of 20 mL of NaOH (1M) were also inserted in the jar in order to trap the CO_2 emitted during each incubation interval, allowing to trap 120 mg CO_2-C kg^{-1} soil. This amount appeared to be satisfactory *a posteriori* since the maximum emission recorded was 93 mg CO_2-C kg^{-1} soil. Similarly, the O_2 concentration calculated in the jar atmosphere never dropped below 11%, ensuring permanent oxic conditions in all incubated soil cores. CO_2 measurements were made at days 3, 7, 14, 28, 42, 56, 70, 84, 98 and 120. The destructive mineral N measurements were carried on 8 replicated soil cores at three dates: day 1, 56 and 120. The moisture content during the incubation, measured *a posteriori*, appeared to be slightly higher than in the disturbed soils: it was 20.8% in the CON system and 21.8% in the CA system on average during the 120 days.

2.5. C, N and microbial biomass

The 16 disturbed soil samples were analyzed for their characteristics, each in triplicate aliquots. Organic carbon and total nitrogen concentration were measured with a CN elemental analyzer (Carlo Erba NA 2000, Milan, Italy) after drying and finely grinding the samples using a ball mill. The microbial biomass-C was determined on field moist samples by fumigation-extraction (Vance *et al.*, 1987), quantifying the soluble C with a Shimadzu TOC-5000/5000A analyzer and a using a 2.2 conversion factor for kc.

2.6. C and N mineralization measurements

The measurement of C mineralization differed between the undisturbed and the disturbed soil samples because of laboratory constraints. The CO_2 emitted in the undisturbed soil samples was trapped into NaOH solution. A solution of $BaCl_2$ (1M) was then added, leading to the formation of a $BaCO_3$ precipitate which was filtered onto a glass fiber filter under vacuum, dried out and weighed. The amount of C mineralized (*mC*) was calculated as follows:

$$mC = mBaCO_3 \; . \; \frac{M_C}{M_{BaCO_3}} \qquad (1)$$

where $mBaCO_3$ is the mass of barium carbonate, M_C and M_{BaCO_3} are the molar masses of C (12 g mol^{-1}) and $BaCO_3$ (197 g mol^{-1}) respectively.

For the disturbed soil samples, an air sample extracted from each incubation jar was injected into a gas chromatograph MICRO-GC (Agilent 3000A, Santa Clara, CA, USA), separating gases according to their molecular weight. The headspace CO_2 concentration was measured by a thermal conductivity detector, expressed in parts per million and converted into mg C. The residual CO_2 in the jar atmosphere was removed at the beginning of incubation and after each measurement by flushing jars with reconstituted CO_2-free air. The agreement between the two methods of measurement of mineralized C was checked successfully in the CON treatment using replicated samples.

The net N mineralization was determined similarly in disturbed and undisturbed soil samples. At each measurement date, the soil samples were taken from the incubation jars, placed in the freezer and thawed subsequently at 4°C during 24h before soil analysis. Mineral N was extracted on a 50 g soil sample after 30 minutes shaking with 100 mL of a KCl solution (1M). The mineral N concentration (ammonium and nitrate) was measured by continuous flow-colorimetry (Skalar Analytical, The Netherlands). Net N mineralization from incubated soil samples was calculated by subtracting the average mineral N content at the start of the incubation from the mineral N content measured at each measurement date.

2.7.Simulation of C and N mineralization kinetics

The C and N mineralization kinetics determined over the 120-days incubation was fitted to a model for each cropping system and each incubation technique (disturbed or undisturbed) separately. On the basis of the results, a linear-exponential model with two pools was used to simulate the absolute and specific C mineralization, along with the C mineralization per unit of microbial C. The cumulative amount of mineralized C was fitted to the "zero-first order" model (Dessureault-Rompré *et al.*, 2016):

$$C = A\,.\,(1 - e^{-k.t}) + B\,.\,t \tag{2}$$

where C is the absolute amount of C mineralized (mg C kg^{-1} soil), A is the rapidly mineralizable pool (mg C kg^{-1} soil), k is its mineralization rate constant (day^{-1}), t is the incubation time (day) and B is the mineralization rate of the slow mineralizing pool (mg C kg^{-1} soil day^{-1}). Equation (2) can also describe the specific mineralization, expressed either per unit of SOC (mg C g^{-1} SOC) or unit of microbial-C (g C g^{-1} Cmic). The optimization of parameters A, B and k was made with the Excel solver tool by minimizing the root mean square error (RMSE) between observed and simulated data:

$$RMSE_j = \sqrt{\frac{1}{n_j}\sum_{i=1}^{n_j}(QC_{ij} - \widehat{QC}_{ij})^2} \tag{3}$$

where n_j is the number of observations in treatment j, QC_{ij} and $\widehat{QC}_{ij}$ are the observed and estimated mineralized C for the observation i and treatment j. The fitting procedure included four steps:

- Step 1: A, B and k were optimized for each plot and for disturbed (optimization O1) and undisturbed (O'1) soil samples.
- Step 2: A and B were optimized for each plot for disturbed (O2) and undisturbed (O'2) soil samples. k was set to the average value found in step 1 for each cropping system.
- Step 3: A and B were optimized for each plot for disturbed (O3) and undisturbed (O'3) soil samples. k was set to the average value found in step 2 for all cropping systems.
- Step 4: it concerned only C mineralization kinetics in undisturbed soil cores (O'4). A and B were optimized for each plot. k was set to the average value found in optimisation O3 for all systems.

N mineralization kinetics was determined on a reduced number of points and appeared to follow a more linear evolution than C kinetics. Therefore, the cumulative N mineralized was described by a simple linear model:

$$N = D\ .\ t \qquad (4)$$

where D is the absolute (mg N kg^{-1} d^{-1}) or specific (mg N g^{-1} SON d^{-1}) mineralization rate.

2.8.Statistical analysis

All the data were analyzed under version 3.5.2 of R (R Core Team, 2018). Given the low number of true replicates in the experiment (two randomized blocks), we considered 8 randomized plots (4 treatments x 2 blocks) with 2 replicates within plots (in the sided subplots) and applied a “nested” analysis of variance, as recommended by Webster and Lark (2018). This ANOVA (corresponding to Table 6 in their paper) was performed to test the effect of the four different cropping systems on several variables: C and N mineralized on day 120, parameters of the mineralization curves, soil C and N contents, C/N ratio and microbial biomass-C at the start of the incubation. We also tested the effect of the previous and current crops on these variables, but did not detect any significant effect on C and N mineralization (data not shown). For disturbed samples, the mean of the three replicates of each subplot and of each variable was used for statistical analysis, *i.e.* four values per cropping system and per date of measurement. For undisturbed samples, the mean of the four soil cores from each subplot was used, *i.e.* four values per cropping system and per date of measurement, except for mineral N at day 0 and day 56, where only one soil core was available for each subplot. The existence of significant effects ($p<0.05$) was followed by a post-hoc comparison test of means with the CLD from the *emmeans* package (Lenth, 2018). The normal distribution of model residues was checked using the Shapiro-Wilk and Levene tests. In case of discordance, the non-parametric test of Kruskal-Wallis was used, followed by means comparison using the kruskal.test from the *agricolae* package.

3. Results

3.1. Soil characteristics

SOC, SON and microbial biomass C (MBC) concentrations measured in the ploughed (or previously ploughed) soil layer in 2016, 18 years after the onset of experiment, did not vary significantly ($p < 0.05$) between three cropping systems but differed in the conservation agriculture system which had higher SOC and SON contents (Table 1). MBC, expressed either in absolute value or relative to organic carbon, tended to be higher in the CA system but the difference was not significant.

3.2. C and N mineralization in disturbed soils

At the end of incubation of disturbed soils (day 120), the cumulative amounts of C mineralized expressed per unit of soil mass did not differ significantly between the four systems, whatever the crop (Figure 2a). The cumulative amounts of N mineralized per unit of soil mass varied more between systems, but not significantly (Figure 2b). The specific mineralization, expressed per unit of SOC (Figure 2c) or SON (Figure 2d) did not differ either between systems. The C mineralization kinetics were satisfactorily modelled with the two pools model, with small RMSEs (Table 2). In optimization O1, the rate constant of the rapidly decomposable pool (k) fitted for each plot was significantly smaller in LI compared to CA, but did not significantly differ between CON and ORG. The size of the rapidly decomposable pool (A) and the mineralization rate of the slow pool (B) did not differ between systems, whatever their unit of expression. Rather similar results were obtained when a single mineralization rate is considered per system (optimization O2), but differences were detected in parameters: *B* was significantly smaller in LI compared to CA and ORG for absolute and specific C mineralization, and *A* was higher in LI than CA for specific C mineralization. Finally, when a common *k* value (0.028 d^{-1}) was used for all systems (optimization O3), the fitted parameters *A* and *B* did not differ significantly between systems, both for absolute and specific C mineralization.

The N mineralization kinetics were well simulated by a linear model, the slope of which did not differ significantly ($p<0.05$) between systems, both for absolute and specific N mineralization (Table 3). It is however noticeable that the treatments CA and ORG seemed to have a greater absolute mineralization rate than the other two cropping systems, consistently with the trend found for C mineralization (Figure 2a). The difference between CA and CON was significant at the 10% probability level. The absence of statistical significance might be due to an insufficient number of replicates.

3.3. C and N mineralization in undisturbed soils

Undisturbed soil cores coming from conservation agriculture plots mineralized significantly more carbon (+53% at day 120) than those from conventional plots (Figure 3). The difference in specific mineralization between the two systems was smaller (+19%) and not significant. The three decomposition parameters calculated independently (optimizations O'1) provided values which did not differ significantly between the two cropping systems (Table 4). This surprising result is attributed to the non linearity of the model and the uncertainty in parameter estimation which is itself due to the correlation between the three parameters. This uncertainty disappeared when a common *k* value was applied to both systems (optimization O'3): in this case, the *A* parameter differed markedly between the two systems for absolute C mineralization (with a much greater value for CA system). A similar difference was found when *k* was set at the value determined in the disturbed cores (k = 0.028 d^{-1}, simulation O'4), with a greater *A* value in the CA than in the CON system. In contrast, the model parameters relative to the specific mineralization kinetics (per unit of SOC) did not differ between systems, except in optimization O'2, where *B* was significantly greater in CA than in the CON system. The N mineralization results showed exactly the same trends than for C mineralization (Table 5). The absolute N mineralization rate was much greater in CA than CON whereas the specific N mineralization rate was similar in both systems. The separation between soil layers indicated that the difference occurred mainly in the two upper layers (0-5 and 5-10 cm). The upper layer mineralized almost three times faster in the conservation than in the conventional system, indicating that the differences in net N mineralization were strongly related to the variations in SON content. A significant difference could be detected in specific N mineralization when considering the entire soil layer (0-27 cm).

It is noticeable that the amounts of C and N mineralized were much larger in undisturbed than in disturbed soil samples (Figure 4). Soil conditioning (disturbed vs undisturbed) has a significant effect on C and N mineralization whereas the cropping system had no significant effect. The relative mineralization ratio of undisturbed *vs* disturbed soil was 1.90 for C and 1.80 for N whereas that of the CA *vs* CON system was 1.06 for C and 1.28 for N.

4. Discussion

4.1.Physical disturbance effect

In incubation studies, the soil is most often incubated after some degree of disruption of the soil structure and sieving in order to decrease the heterogeneity between soil samples. We hypothesized that disturbing the soil prior to incubation would increase the mineralization of C and N because of a de-protection of the organic matter located within the soil structure, and possibly a better oxygen availability in the sieved soil. Indeed, several experiments comparing the net N mineralization in intact soil cores *vs.* sieved soil showed that mineralization rates were increased in sieved soils (Sierra, 1992; Stenger *et al.*, 1995; Ringuelet & Bachmeier, 2002; Li *et al.*, 2013). It is noticeable however that all these studies used the Stanford and Smith's leaching technique which did not provide similar conditions of moisture in disturbed and undisturbed soils. Conversely, other studies studying the effect of physical disturbance on N mineralization (Cabrera & Kissel, 1988; Zhao *et al.*, 2010; Moberg *et al.*, 2013) or C and N mineralization rates (Franzluebbers, 1999; Stenger *et al.*, 2002; Curtin *et al.*, 2014) found little or no impact of disturbance, except the initial flush effect which occurred in disturbed soils which had been dried before the incubation. In our study, we observed an opposite effect of soil disturbance since C and N mineralization rates were higher in undisturbed soil cores. Model fitting showed that the size of the labile pool contributing to C mineralization was much larger in undisturbed soil cores (*e.g.* 565 in CON vs 377 mg C kg^{-1} in CA) when a single mineralization rate was set per system (optimization O'2, Table 4) than in disturbed samples (100 and 90 mg C kg^{-1} respectively) (optimization O2, Table 2). One possible explanation is a higher abundance of labile SOC in undisturbed soil cylinders than in disturbed soils samples that had been sieved to 10 mm and from which visible coarse plant residues and fine roots had been manually eliminated. At the end of incubation, the undisturbed soil cylinders were manually sorted out and were found to contain biological attributes: germinated seeds (average 2.2 per kg soil), insects (1.8), earthworms (1.0) and dead roots (0.7 g DM per kg soil), all having a greater abundance in CA compared to CON. This living or labile organic matter could explain why more C and N were mineralized from undisturbed than disturbed soil samples. If we compare to the results of Pang *et al.* (2012) who studied the impact of earthworms on C and N mineralization, the extra C mineralized in the undisturbed soils might come from the earthworm activity, but the extra N mineralized is greater than the one reported by these authors. A second possible explanation is the higher moisture content in the undisturbed soils which is likely to be more favourable to mineralization. If we apply to our data the relationship established by Paul *et al.* (2003) between N mineralization and water potential, we can estimate that N mineralization at the moisture content found in undisturbed CA soil should have increased mineralization by less than 15% compared to the disturbed CA soil.

4.2. Effect of cropping system on mineralization rates

We observed no significant effect of the cropping system on the absolute rate of C and N mineralization, except for undisturbed soil cores in which the conservation had a higher rate than the conventional system. In fact, there was a consistent trend in having higher rates of C and N mineralization in disturbed soil samples of CA and ORG systems compared to CON and LI, but the difference was not significant at the 5% level. The four systems did not show any difference in their specific rate of mineralization, expressed either *vs* SOC, SON or microbial biomass C. This result is fully consistent with the hypothesis made by Autret *et al.* (2016) who simulated the long-term variation of SOC in the same experiment. The authors found no significant SOC change in the LI and CON systems, a moderate increase in the ORG system and a high increase in the CA system after 17 years. They could simulate satisfactorily the evolution of SOC stocks by accounting for the crop residues inputs using a two pools model and assuming a single mineralization rate constant for all systems. This assumption is confirmed by the present study, both for C and N mineralization and for disturbed and undisturbed soil samples. It suggests that the main driver of the SOC variation among these four cropping systems is the amount and nature of organic inputs (which varied widely between systems) rather than the variation in soil tillage (varying from permanent no till to annual ploughing) which may not affect the specific mineralization rate (Autret *et al.*, 2016).

Several previous studies showed higher mineralization rates of C and N in organic cropping systems than in conventional ones, but these could be explained by the applied organic fertilizers inputs (Gunapala & Scow, 1998; Carpenter-Boggs *et al.*, 2000). Regarding the effects of tillage, most studies focused on the superficial soil layers, e.g. 0-5 cm, and found higher mineralization rates of C and N in no-till soils, easily ascribed to the relative enrichment in organic matter and particulate organic matter content in surface layers of no till soils (*e.g.* Beare *et al.*, 1994). However, when deeper layers were included in the comparison, specific C and N mineralization rates were either similar or even greater in the untilled compared to tilled soils (Beare *et al.*, 1994; Franzluebbers *et al.*, 1995; Oorts *et al.*, 2006; Jacobs *et al.*, 2010).

The similarity in specific mineralization rates observed in the four cropping systems suggests that either the decomposability of the organic matter is similar in the different cropping systems or that the environmental conditions for decomposers are the same. Regarding the decomposability of the organic matter, the nature of organic inputs to soil is rather similar in the different cropping systems since wheat is cropped one year out of two in all systems (Autret *et al.*, 2016). There are slight differences however, as the frequency of pea, rapeseed and alfalfa varied among systems. There was almost no organic fertilization in all treatments. No major differences in the biochemical quality of the residues of the main crops are then expected between the different cropping systems. Regarding the environmental conditions, the soils (layer 0-27 cm) had similar pH (7.20, 7.09, 7.14 and 7.06) and C/N ratio (9.91, 9.94, 9.71 and 9.75 in CON, LI, ORG and CA respectively). However, the very contrasted tillage intensity between systems is expected to affect soil structure and increase the physical protection of organic matter in the no-till soils of CA system (Six *et al.*, 2000). While a more stable aggregated structure was observed early in the CA soil (Balabane *et al.*, 2005), there was no difference with other systems in bulk density of the 0-30 cm layer sampled in 2014 (Autret *et al.*, 2016). In the incubated undisturbed cores, two opposite processes might explain the similar specific mineralization rates of C and N in the CON and CA cylinders: there was more coarse labile organic matter in the CA cores (see above) but their soil structure was less favourable to decomposition. In the case of disturbed soil, sieved to 10 mm, the similarity in C and N mineralization rates over 120 days could be due to either an absence of difference in physical protection among systems, or to the fact that physical protection mainly affects organic matter with a long residence time, longer than that mineralized during the 120 days incubation.

Our results suggest that the observed increased C and N stocks in the conservation agriculture system at La Cage are mainly due to the increased OC inputs and not to a reduction in SOC mineralization rate. However, this conclusion does not fully apply to the organic agriculture system in which the increase in SOC stocks after 17 years was accompanied by smaller OC inputs than in the CON system (Autret *et al.*, 2016) and similar specific mineralization rates as shown here. Previous studies have indicated that cropping systems including a high frequency of alfalfa could maintain or increase SOC stocks in spite of reduced OC inputs (Gregorich *et al.*, 2001; Syswerda *et al.*, 2011; Chang *et al.*, 2012). The specific effect of alfalfa (compared to annual crops) may be attributed to the higher proportion of root material, which is known to result in a higher rate of C stabilization than aerial residues (e.g. Kätterer *et al.*, 2011). Another possible explanation lies in the physiology of microorganisms. Kallenbach *et al.* (2015) recently showed that the carbon use efficiency of soil microorganisms was higher in an organic agriculture trial than in its conventional agriculture reference plot. This was explained by changes in the microbial community structure as well as changes in the physiology of microorganisms in nutrient poor environments. For a given amount of OC inputs to soil, a higher carbon use efficiency would result in more C being stored in soil for an unchanged rate of mineralization. This process requires more investigations in the alternative cropping systems studied here (ORG, LI, CA), all characterized by a reduction in fertilizer inputs to soil and, for ORG and CA, by a higher proportion of legumes being grown either as a cash crop or as a cover crop.

5. Conclusion

The effects of alternative arable cropping systems on soil organic matter can result from modified organic inputs to soil or modified rates of mineralization of C and N from soil. Here we found that alternative arable cropping systems, *i.e.* a legume based organic agriculture system and a conservation agriculture system, which resulted in increased soil carbon storage compared to a conventional system, exhibited similar specific C and N mineralization rates during laboratory incubation. This suggests that the observed increased SOC stocks are due to increased biomass returns to soil or to changes in microbial physiology rather than reduced mineralization. Overall it suggests that increasing organic C returns to soil, as above ground or below ground plant material is an efficient strategy to increase soil organic carbon stocks, both to maintain and increase soil quality and to mitigate climate change. For the latter objective a full greenhouse gas balance is needed for alternative arable cropping systems.

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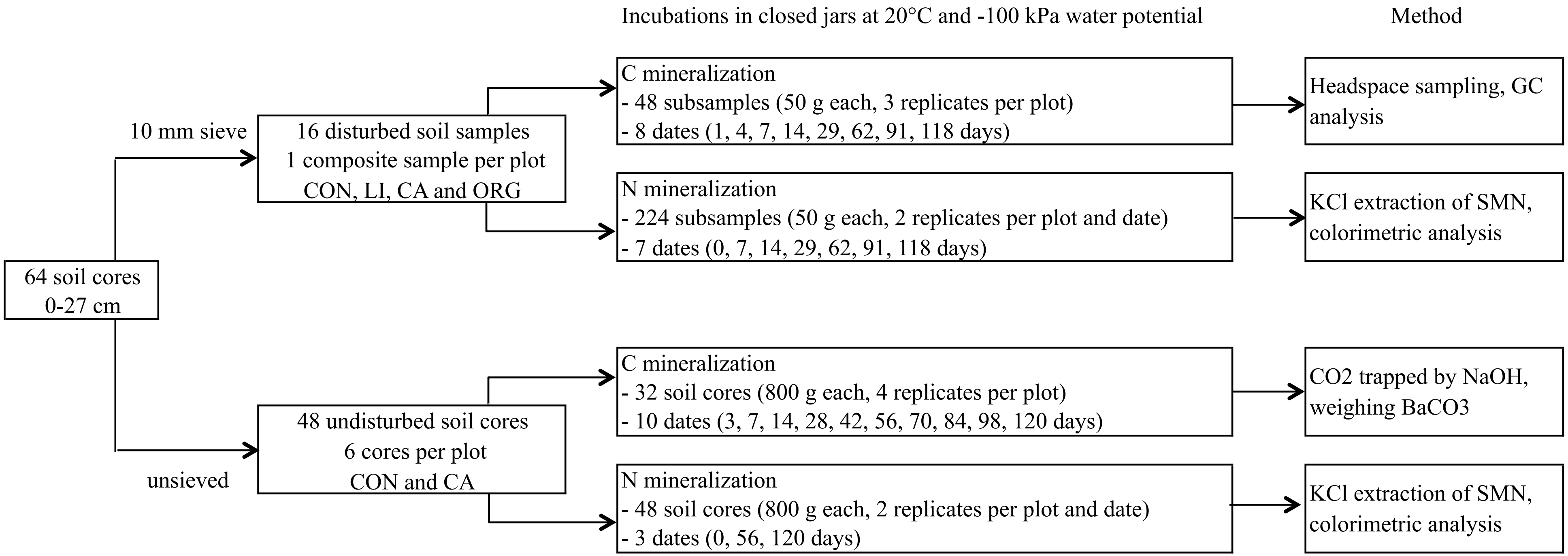
Incubations in closed jars at 20°C and -100 kPa water potential
Method
64 soil cores
0-27 cm
10 mm sieve
16 disturbed soil samples
1 composite sample per plot
CON, LI, CA and ORG
C mineralization
- 48 subsamples (50 g each, 3 replicates per plot)
- 8 dates (1, 4, 7, 14, 29, 62, 91, 118 days)
Headspace sampling, GC analysis
N mineralization
- 224 subsamples (50 g each, 2 replicates per plot and date)
- 7 dates (0, 7, 14, 29, 62, 91, 118 days)
KCl extraction of SMN, colorimetric analysis
unsieved
48 undisturbed soil cores
6 cores per plot
CON and CA
C mineralization
- 32 soil cores (800 g each, 4 replicates per plot)
- 10 dates (3, 7, 14, 28, 42, 56, 70, 84, 98, 120 days)
CO2 trapped by NaOH, weighing BaCO3
N mineralization
- 48 soil cores (800 g each, 2 replicates per plot and date)
- 3 dates (0, 56, 120 days)
KCl extraction of SMN, colorimetric analysis

Figure 2. C and N mineralization kinetics in disturbed soil samples: a, b) per kg of soil; c, d) per g of SOC or TN; e) per mg of microbial biomass C. Dots are observed values and full lines represent the fitted curves of C and N mineralization (optimization O3, see Table 2).
Vertical bars represent the mean confidence intervals ($p<0.05$) at the end of incubation.

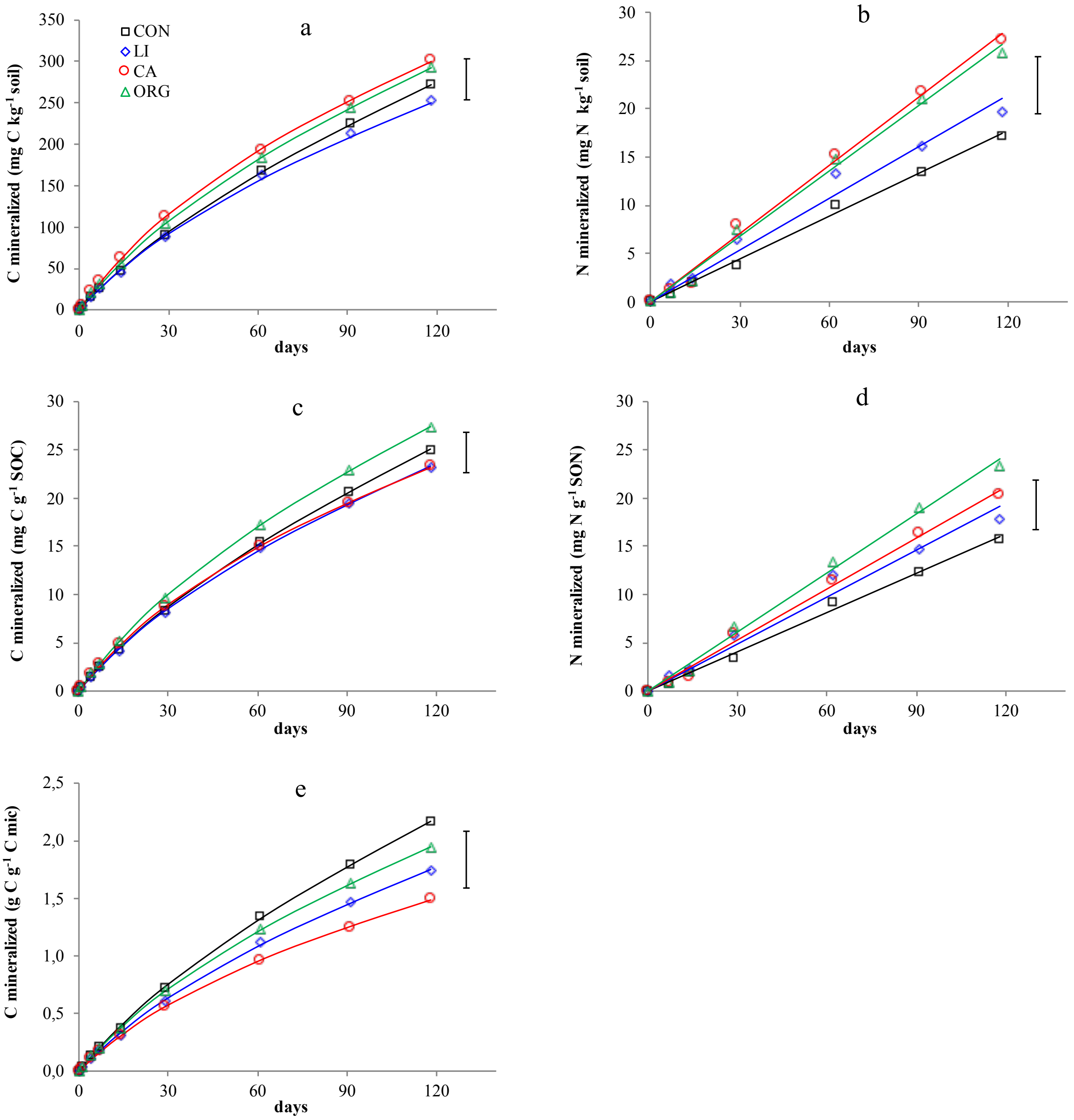

Figure 3. C mineralization kinetics in undisturbed soil samples: a) per kg of soil; b) per g of SOC.
Dots are observed values and full lines represent the fitted curves of C mineralization (optimization O'4, see Table 5).
Vertical bars represent the mean confidence intervals ($p<0.05$).

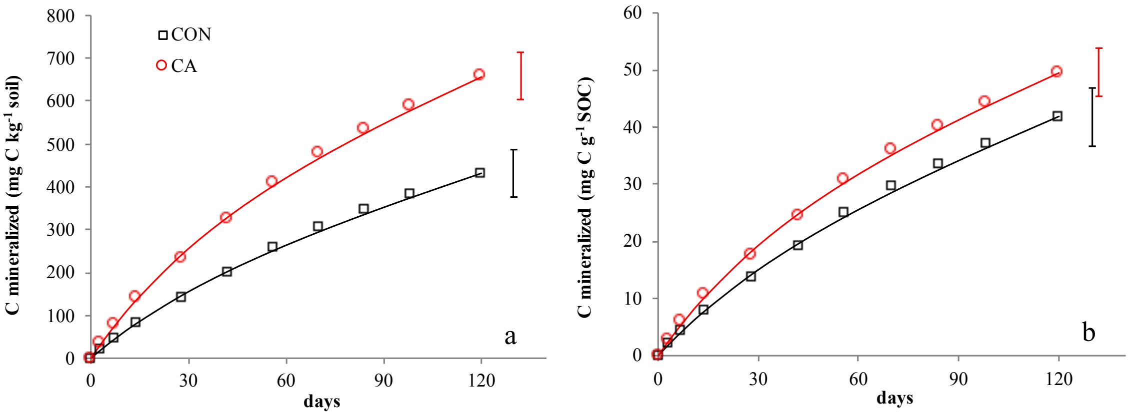

Figure 4. Specific amounts of C and N mineralized in disturbed and undisturbed soil samples of CON and CA systems at the last day of incubation (day 120).
Bars represent the standard deviations.

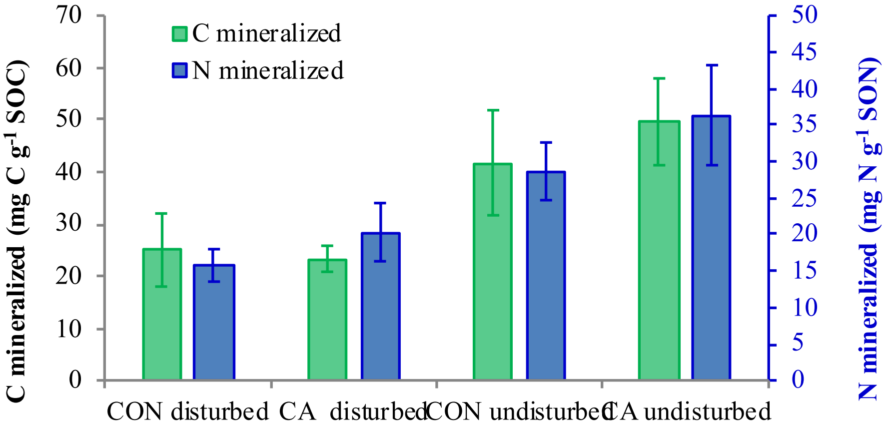

Table 1 Soil organic C (SOC), total N (SON) and microbial biomass C (MBC) concentrations measured in disturbed soils (0-27 cm). Values in brackets are standard deviations. Letters indicate significant differences between cropping systems ($p < 0.05$).

Cropping system	SOC			SON			MBC			MBC/SOC		
	g kg^{-1}						mg C kg^{-1}			%		
CON	10.9	(1.2)	b	1.10	(0.12)	b	127	(11)	a	1.2	(0.1)	a
LI	10.8	(1.5)	b	1.09	(0.14)	b	155	(50)	a	1.4	(0.3)	a
CA	13.0	(1.6)	a	1.33	(0.15)	a	202	(20)	a	1.6	(0.1)	a
ORG	10.7	(0.5)	b	1.10	(0.05)	b	151	(14)	a	1.4	(0.2)	a

Table 2 Optimized parameters (*A*, *B* and *k*) obtained in curve fitting to the C mineralization model (eq. 2) of disturbed soil samples. Values in brackets are standard deviations. Letters indicate significant differences ($p < 0.05$) between cropping systems (or rows).

	cropping system	mineralization rate constant	absolute C mineralization			specific C mineralization (per unit of SOC)			specific C mineralization (per unit of MBC)		
		k	*A*	*B*	RMSE	*A*	*B*	RMSE	*A*	*B*	RMSE
		d^{-1}	$mg\ kg^{-1}$	$mg\ kg^{-1}\ d^{-1}$	$mg\ kg^{-1}$	$mg\ g^{-1}$ SOC	$mg\ g^{-1}$ SOC d^{-1}	$mg\ g^{-1}$ SOC	$g\ g^{-1}$ MBC	$mg\ g^{-1}$ MBC d^{-1}	$g\ g^{-1}$ MBC
O1*	CON	0.021 (0.010) *ab*	150 (103) *a*	1.3 (0.5) *a*	5.3	12.3 (6.2) *a*	0.13 (0.04) *a*	0.5	1.16 (0.72) *a*	10.6 (4.5) *a*	0.04
	LI	0.019 (0.005) *b*	153 (59) *a*	1.0 (0.4) *a*	6.4	14.8 (7.1) *a*	0.09 (0.03) *a*	0.6	1.14 (0.76) *a*	6.7 (2.6) *a*	0.04
	CA	0.039 (0.006) *a*	92 (10) *a*	1.8 (0.3) *a*	4.0	7.1 (0.9) *a*	0.14 (0.02) *a*	0.3	0.46 (0.06) *a*	8.8 (1.1) *a*	0.02
	ORG	0.028 (0.012) *ab*	135 (86) *a*	1.5 (0.3) *a*	6.5	12.5 (7.9) *a*	0.14 (0.03) *a*	0.6	0.88 (0.54) *a*	10.1 (2.5) *a*	0.04
O2*	CON	0.022 (0.010) *ab*	100 (48) *a*	1.5 (0.3) *ab*	6.1	9.2 (4.6) *ab*	0.14 (0.03) *a*	0.6	0.81 (0.45) *ab*	12.0 (4.5) *a*	0.05
	LI	0.018 (0.005) *b*	146 (27) *a*	1.0 (0.3) *b*	6.8	14.0 (1.0) *a*	0.09 (0.03) *b*	0.6	0.83 (0.54) *ab*	8.8 (2.6) *a*	0.10
	CA	0.039 (0.006) *a*	90 (17) *a*	1.8 (0.4) *a*	5.0	6.9 (1.8) *b*	0.14 (0.01) *a*	0.4	0.45 (0.11) *b*	8.9 (1.1) *a*	0.03
	ORG	0.032 (0.012) *ab*	90 (24) *a*	1.7 (0.2) *a*	7.9	8.4 (1.9) *ab*	0.16 (0.02) *a*	0.7	0.60 (0.18) *a*	11.6 (2.5) *a*	0.05
O3*	CON	0.028 (0.011)	79 (38) *a*	1.7 (0.4) *a*	6.5	7.3 (3.7) *a*	0.15 (0.03) *a*	0.6	0.63 (0.35) *a*	13.2 (3.5) *a*	0.05
	LI	0.028 (0.011)	88 (17) *a*	1.4 (0.3) *a*	8.0	8.1 (0.5) *a*	0.13 (0.03) *a*	0.8	0.60 (0.13) *a*	10.0 (4.1) *a*	0.06
	CA	0.028 (0.011)	123 (22) *a*	1.5 (0.4) *a*	7.7	9.5 (2.4) *a*	0.12 (0.02) *a*	0.6	0.61 (0.15) *a*	7.6 (1.0) *a*	0.04
	ORG	0.028 (0.011)	102 (27) *a*	1.6 (0.2) *a*	8.1	9.5 (2.1) *a*	0.15 (0.03) *a*	0.8	0.68 (0.20) *a*	10.9 (1.3) *a*	0.05

* O1: A, B and k optimized for each plot

* O2: A, B and k optimized for each plot, with a common k per system

* O3: A, B and k optimized for each plot, with a common k for all systems

Table 3 Absolute and specific N mineralization rates (parameter D in eq. 4) in disturbed soil samples. Values in brackets are standard deviations. Values in brackets are standard deviations. Letters indicate significant differences ($p < 0.05$) between cropping systems (or rows).

cropping system	absolute N mineralization rate			specific N mineralization rate (per unit of SON)		
	mg N kg^{-1} d^{-1}			mg N g^{-1} SON d^{-1}		
CON	0.15	(0.02)	a	0.13	(0.03)	a
LI	0.17	(0.02)	a	0.15	(0.02)	a
CA	0.23	(0.07)	a	0.18	(0.07)	a
ORG	0.22	(0.03)	a	0.20	(0.03)	a

Table 4 Optimized parameters (*A*, *B* and *k*) obtained in curve fitting to the C mineralization model (eq. 2) of undisturbed soil samples. Values in brackets are standard deviations. Letters indicate significant differences ($p < 0.05$) between cropping systems (or rows).

optimization procedure	cropping system	mineralization rate	absolute C mineralization			specific C mineralization (per unit of SOC)		
		k d^{-1}	*A* $mg\ kg^{-1}$	*B* $mg\ kg^{-1}\ d^{-1}$	RMSE $mg\ kg^{-1}$	*A* $mg\ g^{-1}$ SOC	*B* $mg\ g^{-1}$ SOC d^{-1}	RMSE $mg\ g^{-1}$ SOC
O'1	CON	0.014 (0.009) *a*	484 (299) *a*	0.8 (1.1) *a*	3.7	47 (30) *a*	0.07 (0.10) *a*	0.36
	CA	0.021 (0.009) *a*	462 (194) *a*	2.2 (1.3) *a*	5.2	34 (13) *a*	0.17 (0.10) *a*	0.39
O'2	CON	0.010 (0.000)	565 (189) *a*	0.3 (0.4) *b*	4.1	52 (17) *a*	0.04 (0.04) *b*	0.39
	CA	0.021 (0.000)	377 (44) *a*	2.5 (0.3) *a*	5.9	28 (4) *a*	0.19 (0.02) *a*	0.44
O'3	CON	0.017 (0.000)	246 (83) *b*	1.8 (0.2) *a*	4.4	24 (8) *a*	0.17 (0.03) *a*	0.43
	CA	0.017 (0.000)	473 (55) *a*	2.0 (0.3) *a*	6.0	36 (5) *a*	0.15 (0.02) *a*	0.45
O'4	CON	0.028 (0.000)	145 (48) *b*	2.4 (0.4) *b*	5.3	14 (4) *a*	0.24 (0.05) *a*	0.52
	CA	0.028 (0.000)	280 (34) *a*	3.2 (0.3) *a*	6.3	21 (3) *a*	0.24 (0.02) *a*	0.47

O'1: *A*, *B* and *k* optimized for each plot
O'2: *A*, *B* and *k* optimized for each plot, with a common *k* per system
O'3: *A*, *B* and *k* optimized for each plot, with a common *k* for CON and CA
O'4: *A* and *B* optimized for each plot, with *k* determined previously in O3

Table 5 Absolute and specific N mineralization rates in each layer of undisturbed soil cores (mean rate measured in 15 replicated cores during 120 days). Values in brackets are standard deviations. Letters indicate significant differences in each layer between the two cropping systems ($p<0.05$).

cropping system	Soil layer	absolute N mineralization rate			specific N mineralization rate (per unit of SON)		
	cm	mg N kg^{-1} d^{-1}			mg N g^{-1} SON d^{-1}		
CON	0-5	0.27	(0.06)	a	0.25	(0.06)	a
	5-10	0.21	(0.05)	a	0.19	(0.04)	a
	10-27	0.22	(0.06)	a	0.20	(0.06)	a
	0-27	0.23	(0.04)	a	0.24	(0.03)	a
CA	0-5	0.75	(0.21)	b	0.32	(0.09)	a
	5-10	0.49	(0.14)	b	0.31	(0.08)	a
	10-27	0.31	(0.06)	a	0.26	(0.06)	a
	0-27	0.41	(0.08)	b	0.30	(0.06)	a