Sensitivity analysis of C and N modules in biogeochemical crop and grassland models following manure addition to soil

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Summary

Process-based crop and grassland models estimating carbon (C) and nitrogen (N) dynamics are widely used to investigate best management practices in agriculture. They integrate several processes in a complex structure, but studies where modules corresponding to specific processes extracted from the whole model structure are assessed independently are uncommon. With the support of documented aerobic incubation trials in manure-amended soils, a sensitivity analysis was performed on the C–N cycling processes of four modules (MOD1–4), corresponding to the models APSIM, EPIC, FASSET and STICS. The results show that the parameter, ’substrate use efficiency’, had the most effect on the predicted values of net CO$_2$ emissions and net N mineralization, together with the C/N ratio of the soil microbial biomass. They explained 74–75% on average of both output variances, whereas parameters determining manure C and N partitioning and first-order decomposition constants of manure pools explained, on average, an additional 17–19%. Efforts should be focused on calibrating these parameters for more accurate simulations. The greater sensitivity of both outputs to parameters related to manure pools in more complex modules (MOD2–4) facilitates their adaptation to specific contexts, whereas MOD1 probably requires that parameters related to soil pools are also adapted to specific applications. Parameter interactions were limited, becoming noticeable only in situations of N-limited soil organic matter decomposition. Models MOD1 and MOD 3 allowed soil microbial biomass to vary its C/N ratio temporarily, and therefore were less sensitive to mineral N availability and more easily adapted to a wide range of situations. This study provides essential information to support the development of state-of-the-art biogeochemical models.

Highlights:

- We compared four C–N modules embedded in process-based biogeochemical models.
- We used sensitivity analysis to assess the simulation of manure decomposition in soil.
- We identified a few parameters that influenced CO$_2$ emissions and N mineralization.
- We found that substrate use efficiency explained most of the output variance for all models.
Keywords

Animal slurry; CO$_2$ emissions; N mineralization; parameter calibration; soil organic matter.
Introduction

Complex dynamic agricultural and environmental models enable carbon (C) and nitrogen (N) fluxes in terrestrial ecosystems to be simulated. The simulations carried out with these models are important for estimating greenhouse gas emissions, crop productivity and leaching losses of nitrate-N under various pedo-climatic conditions and different N management scenarios. Modules (sub-models) describing the changes in C and N pools of soil and of added organic materials are essential components of these models.

In C–N modules, plant material and animal manures are generally modelled to enter the soil environment as readily decomposable (carbohydrate-like) and resistant (lignin and cellulose-like) materials (Henriksen et al., 2007; Izaurralde et al., 2006; Petersen et al., 2005b; Probert et al., 2005). The soil component consists of different pools of various residence times, varying from months for labile products of microbial decomposition to thousands of years for organic substances with strong organic–mineral bonds (Dungait et al., 2012; Falloon & Smith, 2000). There are considerable differences in the understanding and interpretation of soil organic matter (SOM) processes in current C–N modules, both regarding number of pools and type of decomposition kinetics used to represent SOM turnover (Manzoni & Porporato, 2009). Equations implemented in C–N modules often require a large number of parameters, defined as quantities that remain constant within a simulation but can vary among different simulations. Typically, in C–N modules parameters are quantities related to soil or quantities related to added substrates like animal manure. The values of simulated state variables (e.g. CO₂ emissions and soil mineral N concentration) at different points in time are the model outputs of interest for the users.

Simulated C and N transformations are generally strongly nonlinear and interact with other processes (e.g. water flows and plant growth) simulated in agricultural and environmental models. Moreover, the uncertainty associated with the estimates of several parameter values increases the risk of inaccuracies in model predictions. For these reasons, results of alternative state-of-the-art terrestrial
biogeochemical models, describing the contribution of agricultural systems to C and N sequestration and source or sink status, might diverge significantly (Brilli et al., 2017). It is therefore important to compare the predictions from C–N modules characterized by different structures separately from other sources of uncertainty that arise from processes simulated in the larger models in which the C–N modules are embedded.

An area where C–N modules is particularly important is animal manure management because of complex and dynamic manure–soil interactions. Liquid and solid manure application is a common practice in livestock systems, which provides an input to soils of heterogeneous materials made of decomposable compounds (in the range from slow to fast). These compounds include volatile fatty acids, colonic cells, ruminal bacteria and more resistant compounds in different proportions (Morvan & Nicolardot, 2009; Van Kessel et al., 2000). The C to organic N ratio (C/ON; g C g N⁻¹) of the added materials and the availability of inorganic N in the soil are two important interacting factors that profoundly affect C and N cycling following manure addition to soil. Previous aerobic incubation studies (Bechini & Marino, 2009; Morvan et al., 2006; Thomsen & Olesen, 2000) have shown the complexity of responses related to C respiration and N immobilization in manure-amended soils. The suitability of simulation models to reproduce, through adequate model parameterization, C and N decomposition dynamics after manure incorporation has also been shown to be difficult (Cavalli & Bechini, 2012; Gillis & Price, 2016; Petersen et al., 2005b; Probert et al., 2005).

Determining parameter values from field and laboratory measurements or calibration may require considerable effort and costs. Therefore, a sensitivity analysis might help to identify key model parameters involved in the simulation of relevant C–N processes prior to using models to assess the effects of field practices. In conventional practice, sensitivity analysis carried out before calibration reduces the number of parameters subject to calibration. In this way, optimized sets of parameters can be identified better, either specific for soil and substrate-type (Cavalli & Bechini, 2011; Gillis & Price, 2016; Petersen et al., 2005b) or common to all soils (Petersen et al., 2005a).
Sensitivity analysis provides reliable advice only if the uncertainties of the simulated outputs match the statistical distributions of the measured variables that the output represents. For this reason, the choice of the statistical distributions of model parameters is a critical issue for the analysis (Pianosi et al., 2016). The statistical distributions of some parameters can be derived from measured properties of soil and manure, but those of others must be obtained by calibration. The research described here sets up a procedure that first calibrated the statistical distribution of parameters to fit the distribution of simulated outputs to a measured distribution taken from a reference data set. Then, the sensitivity analysis was done with the statistical distributions of the calibrated parameters. With the support of published results of aerobic incubation trials, this study compared four modelling approaches (C–N modules) with global sensitivity analysis to soil C and N cycles (differing in the number of parameters, pools and fluxes), applied to the decomposition of animal manures. The four modelling approaches were selected for this study because they are representative of the mechanistic models reported in the literature (Campbell & Paustian, 2015; Falloon & Smith, 2000; Manzoni & Porporato, 2009; Stockmann et al., 2013), and characterized by different structures and principles governing C and N turnover in soil.

Material and methods

First, we provide a brief description of the selected C–N modules, directing readers to original papers for the full details. Then, we describe the procedure used to calibrate model parameters in order to match the variation in net CO₂ emissions and net N mineralization as measured in manure amended-soils by laboratory incubation experiments (Figure 1a–c). Finally, we provide details about the procedure used to perform sensitivity analysis of model outputs for the chosen modules (Figure 1d).

C and N modules

Description and implementation
Four SOM models (MOD1–4; Figure 2) were derived from the C–N modules of the models STICS (Brisson et al., 2009), APSIM (Probert et al., 1998, 2005), EPIC (Izaurralde et al., 2006) and FASSET (Berntsen et al., 2003; Petersen et al., 2005a, 2005b).

All of the above models use interconnected multiple pools to simulate C and N dynamics. These models differ in structure (number of key pools and fluxes) and total number of parameters (Table 1). In addition, the C to N ratio (C/N; g C g N⁻¹) in the microbial biomass of STICS (MOD1) and in all the native pools of EPIC (MOD3) varies according to mineral N availability (Izaurralde et al., 2006) and microbial biomass N requirement during SOM decomposition (Brisson et al., 2009; Giacomini et al., 2007). In contrast, C/N is set to fixed values in the pools of APSIM (MOD2) and FASSET (MOD4). Finally, modules implement different response functions to external factors such as soil temperature, soil moisture and, for MOD3 and MOD4, texture. All models assume first-order decomposition kinetics and represent soil microbial biomass explicitly. In our implementation, some minor changes were made to the EPIC module (Table S1; Supporting Information).

**Model outputs and initialization**

Parameter calibration and sensitivity analysis were performed on two model outputs: accumulated net CO₂ emissions and net N mineralization (NNM). The units were mg C 100 mg⁻¹ manure C and mg N 100 mg⁻¹ manure C, respectively, to ensure consistency with the published data. We obtained net CO₂ emissions from total CO₂ emissions by subtracting the amount of CO₂ emitted from unamended soil. The NNM represents the variation in soil mineral N after subtracting the amount of N in unamended soil compared to day 0 (Bechini & Marino, 2009). Positive NNM indicates net mineralization of organic N (ON) while negative values indicate net immobilization of soil mineral N. The initialization process performed during parameter calibration and sensitivity analysis is reported in Table S2 (Supporting Information).

**Parameter calibration**

**Reference datasets**
A reference distribution of net CO$_2$ emissions and NNM data (Figure 1a) was obtained by taking these variables from 17 published aerobic incubation experiments (Table S3; Supporting Information). Different manure types were considered (faeces, solid manures, liquid manures), also with respect to animal species (bovine, pig, sheep and poultry), manure storage systems and treatment alternatives (fresh, anaerobically stored, anaerobically digested, separated fractions). Treatments belonging to compost-amended soils were excluded.

We recognize that the variation in measured C respiration and NNM was not due to manure type only, because different incubation conditions (soil type and temperature) were used in the experiments considered. However, all the above citations (Table S3; Supporting Information), except Thomsen & Olesen (2000) and Thomsen et al. (2003), disrupted soil structure by soil sieving (usually through 2- or 4-mm mesh size), thus reducing the effect of soil type on C and N turnover. Moreover, because the influence of soil type was small (even in the case of undisturbed soil), we assumed that all the variation measured originated from manure properties.

We calibrated, separately for each treatment, two empirical models to estimate measured variables at fixed dates because different dates of sampling among experiments made it impossible to compare them directly. We used a double exponential model (Bechini & Marino, 2009) for net CO$_2$ measurements and a Monod-type three-parameter model (Peters & Jensen, 2011) for NNM. Thereafter, two datasets (one for net CO$_2$ emissions and one for NNM) were created with measured or simulated values at days 3, 7, 14, 28, 42, 84, 120 and 180 to obtain the frequency distributions of the reference output at different stages of manure decomposition.

Parameter distributions

The statistical distributions of model parameters (Figure 1b) were derived from a literature review of manure, soil and microbial biomass properties, and from model documentation (Table 1). We linked the partitioning of manure C and ON to the results of proximate analysis (Henriksen et al., 2007; Morvan et al., 2006; Peters & Jensen, 2011). In this way, manure pools comprised a labile (fast-
decomposable, soluble-like) pool (ML), a resistant (cellulose-like) pool (MR) and a very resistant (lignin-like) pool (MVR) (Figure 2; Table 1). Either uniform or normal distributions were considered, and for each parameter the one that approximated the data distribution best was chosen. Alternatively, first-order decomposition constants were arbitrarily assigned a normal distribution with mean $\mu$ equal to the reported reference value, and a standard deviation $\sigma$ equal to $0.25\mu$. The distributions of all model parameters were bounded between a lower and an upper limit of $\pm 3\sigma$, respectively.

Calibration procedure for parameters

When the model was run with the initial distributions of parameters taken from the literature, relevant deviations were observed between the distributions of simulated and reference outputs. Therefore an ad hoc procedure (Figure 1c) was conceived to adjust the distributions of simulated outputs to the measured ones. As a result, a revised distribution was obtained for some parameters (Table 1). These were the decay constants of microbial biomass ($K_{SMB}$ for models MOD1–3; $T_{SMB2}$ in MOD4) and of manure pools ($K_{MAN}$ in MOD1, $K_{ML}$ and $K_{MR}$ in MOD3 and MOD4, and $K_{ML}$, $K_{MR}$ and $K_{MVR}$ in MOD2). The optimization procedure (Figure S1; Supporting Information) was designed to find new values iteratively of $\mu$ for these parameter distributions that minimized the root mean squared error (RMSE) between observed and simulated output frequencies of net CO$_2$ emissions and NNM at the eight dates in the two reference datasets. This task was achieved by a local search method (Nelder & Mead, 1965) coupled with a global optimization genetic algorithm OMNIOPT (Deb & Tiwari, 2008) to reduce the risk of obtaining sub-optimal values of $\mu$ because of issues of local minima. With the adopted procedure, for each new value of $\mu$ found by the Nelder–Mead algorithm, $\sigma$ and the lower and upper limits ($\pm 3\sigma$) were recalculated for the decay constants. Thereafter, the model was run repeatedly (2048 times) and all model parameters were sampled from their distributions and frequency distributions of simulated net CO$_2$ emissions, and NNM values were assessed.

Preliminary calibration provided values for the decomposition constants of microbial biomass that produced too large a turnover of the pool (in practice the size of the microbial biomass pool remained
close to zero). To avoid this, and because the reference data set did not include measurements of SMB, we decided to constrain the size of the SMB pool to ±50% of a reference time series (expressed as % manure C) obtained by Marino P. (University of Milan, Italy; personal communication). This was from an 180-day aerobic incubation experiment with a loamy soil amended with maize (*Zea mays* L.) stalks, pig slurry and ammonium sulphate.

### Sensitivity analysis

#### The method of Sobol

Global sensitivity analysis methods evaluate the effect of a model parameter on given outputs, whereas all other model parameters are varied simultaneously, thus accounting for interactions among them without depending on stipulation of a nominal point as they explore the entire range of each parameter. The method of Sobol (Saltelli *et al*., 2010) relies on the decomposition of the total model output variance (*V*) that, for *w* independent model parameters (input factors), provides $2^w - 1$ terms of increasing order (1–*w*), i.e. partial conditional variances from single parameters ($V_i$) and interactions among them ($V_{ij}$, $V_{ijm}$, and so on):

$$V = \sum_i V_i + \sum_{i<j} V_{ij} + \sum_{i<j<m} V_{ijm} + \ldots + V_{12\ldots w},$$

[Eq. 1]

where *i*, *j*, *m* and *w* represent model parameters.

Sensitivity indices (*S*) are calculated as the ratios of conditional variances ($V_i$, $V_{ij}$, etc.) to total (unconditional) variance ($S_i = V_i/V$; $S_{ij} = V_{ij}/V$, and so on), and quantify portions of the total variance that is due to variation of single parameters or a combination of them (Saltelli *et al*., 2010).

In general, accurate estimation of sensitivity indices requires several model runs. To reduce computational time, only first-order effects ($S_i$), and not higher-order terms, are usually calculated. Instead, interactions among model parameters can be taken into account by calculating total-order indices ($ST_i$), representing the whole contribution of parameters (alone and in combination with all other parameters) to total output variance. Indeed, $ST_i$ is the sum of all terms in Equation (1) involving...
parameter \( i \), divided by \( V \). The quantity \( ST_i - S_i \) provides an estimate of the portion of total variance due to all interactions (from order two to order \( w \)) between the \( i^{th} \) parameter and all other parameters. Quasi-Monte Carlo estimation of first- and total-order sensitivity indices were implemented according to Saltelli et al. (2010) using a sample size of \( 2^{16} \). The Sobol sequence generator was initialized with the set of direction numbers provided by Joe & Kuo (2008).

**Modelling scenarios during sensitivity analysis**

Sensitivity analysis (Figure 1d) was executed by varying parameter values using the distributions obtained after calibration (Table 1). To evaluate the sensitivity of model outputs in a broad set of situations representative of possible model applications, we repeated the analysis for the 48 combinations of eight dates ● three manure C/ONs ● two initial soil mineral N contents. The eight dates corresponded to those when reference measurements were available. Selected manure C/ON values were 8, 21 and 34 (the average and \( \pm 2\sigma \) of reference manure C/ON; Table 1), whereas the initial soil mineral N contents were 0.1 mg N kg\(^{-1}\) (to introduce N limitation on C decomposition for large C to N inputs) and 100 mg N kg\(^{-1}\) (to avoid any limiting effect of N on C decomposition).

**Results**

**Parameter calibration**

**Reference datasets**

Net CO\(_2\) emissions from manure-amended soils occurred at exponentially decreasing rates over time (Figure S2a; Supporting Information). After six months, net CO\(_2\) losses averaged 55% of manure C but variation was large, with total C respiration in the range 20–100% of manure C. Nitrogen dynamics differed among treatments. In fact, some manures always induced net N immobilization, whereas others showed net N release from the first days of decomposition (Figure
On average, manures promoted marked net N immobilization in the first week following their addition to soil (2.4 mg N 100 mg manure C$^{-1}$, corresponding to 67% of manure ON). After day 7, net mineral N concentration in soil started to increase and, after six months, NNM averaged 0.6 mg N 100 mg manure C$^{-1}$ (5% of manure ON).

Reference frequency distributions of net CO$_2$ emissions and NNM are shown in Figure 3, limited to the most relevant dates. These dates included the peak of negative NNM (day 7), the switch date from faster to slower rates of net CO$_2$ emissions (day 14) and four additional dates exploring short–medium term decomposition dynamics (days 28, 42, 84 and 180).

Uncertainty in simulated outputs

The initial distribution of model outputs differed from that of the reference data sets for all models (Figure S3; Supporting Information). The calibration procedure effectively improved the simulation of both outputs for all models for most of the dates, which was confirmed by an average decrease in RMSEs by 6 and 4% for net CO$_2$ and NNM, respectively (Table S4; Supporting Information). After calibration, the RMSEs for net CO$_2$ (on average 8%) were smaller than for NNM (on average 21%). There were larger errors (22–34%) at the end of the incubation period, when all models tended to underestimate NNM (Figure 3).

Calibrated parameters

When multiplied by model-specific modifier factors for the simulated soil (depending on clay content, temperature and moisture), the values of decomposition constants were similar in different model pools of similar physicochemical meaning (Figure 4). Calibration provided the following mean residence time (MRT, calculated as the inverse of the first-order decomposition constant) of manure pools for models MOD2–4: 5, 201–252 (on average 228) and 213–2986 (on average 1156) days for the labile (ML), resistant (MR) and very resistant (MVR) pools, respectively. Conversely, optimized average MRT of the manure pool of MOD1 was 42 days. The MRT of soil microbial biomass did not
vary greatly among models; it ranged between 16 and 52 days (29 days on average). Finally, MRT of
the stable soil pools (HUM, SLOW, PASS and NOM; Table 1) was in the order of years (72 years on
average).

Sensitivity analysis

Sensitivity indices under non N-limited C decomposition

In absence of N limitation on C decomposition (large initial soil mineral N), manure C/ON did not
affect the sensitivity indices of all dates and for all models; for this reason, we report them for the
case of manure C/ON equal to 21 only (Figure 5). The sum of first-order indices was larger than 90%
for all dates and models (Figures 5), indicating that both outputs were almost linearly dependent on
parameters, and that higher-order terms (interactions) were negligible.

The substrate use efficiency (Eff) was the most important parameter, with first-order indices in the
range 37–98 and 38–95% for net CO$_2$ emissions and NNM, respectively. The C/N of microbial
biomass ($CN_{SMB}$ for MOD1–3; $CN_{SMB2}$ for MOD4) also contributed considerably to the variation in
NNM (0–39% of NNM variance). However, while sensitivity of NNM to Eff increased over time
(with the exception of MOD4), the opposite occurred for the C/N of microbial biomass whose
sensitivity indices became zero after days 42–84.

A smaller fraction of output variance (2–58 and 0–25% for net CO$_2$ emissions and NNM,
respectively) was due to variation in parameters related to manure pools. The most important ones
were the fraction of manure C assigned to the resistant pool ($FC_{MR}$) and, solely for MOD4, of manure
C and ON assigned to the manure very resistant pool ($FC_{MVR}$ and $FN_{MVR}$). In addition, both outputs
were sensitive to variation in the decomposition constant of manure pools ($K_{MAN}$ for MOD1, $K_{ML}$ for
MOD2–4 and $K_{MR}$ for MOD4). The decay constant of the labile manure pool ML ($K_{ML}$) contributed
substantially to the output variance, mainly in the short term until the size of the pool became
negligible. Sometimes, short-term effects of some other parameters were due to their indirect effects
on the turnover of the ML pool. This was the case of $FC_{MR}$ (Figure 5), which is indirectly related to
the amount of C in ML and, as a consequence, to short-term C and N dynamics. Finally, in MOD4, parameters associated with slow-decomposing pools (e.g. $K_{MR}$; $FC_{MVR}$ and $FN_{MVR}$) had an effect that increased with time because model pools did not completely decompose in the period considered and, as the effect of other parameters diminished, they contributed to a larger fraction of the output variances.

Sensitivity indices under N-limited C decomposition

With small initial soil mineral N (0.1 mg N kg$^{-1}$), sensitivity indices varied depending on manure C/ON. The application of manure with a C/ON ratio of 8 rapidly increased soil mineral N concentration, thus preventing N limitation and resulting in sensitivity indices equal to those obtained in the absence of N limitation for all models (not shown). Nitrogen limitation occurred with manure C/ON ratios of 21 and 34; in this case, sensitivity analysis identified the same set of important parameters determined previously in the absence of N limitation. However, first-order sensitivity indices were usually smaller (Figure 6a; Figure S4a in Supporting Information) while interactions increased (Figure 6b; Figure S4b in Supporting Information). In MOD3, for both simulated outputs, and in MOD1 for net CO$_2$ only, interactions became evident only after the addition of the manure with the largest C/ON (34), whereas in MOD2 and MOD4 they were evident with a C/ON ratio of 21. For all models, interactions among parameters, whenever present (Figure 6b; Figure S4b in Supporting Information), were mainly relevant in the first period of manure decomposition (days 42–84) when they represented up to 37 and 46% of net CO$_2$ emissions and NNM variances, respectively.
**Discussion**

**Simulation of net CO$_2$ emissions and net N mineralization**

Calibrated models satisfactorily simulated experimental distributions of both variables on many dates (Figure 3), ensuring that subsequent sensitivity analyses fully resolved the effects of parameters on reliable time-dependent outputs (Pianosi et al., 2016). Larger errors for NNM (average RMSE = 21%) than for net CO$_2$ (average RMSE = 8%), especially after day 84 (Table S4; Supporting Information), suggest that model improvements are required to simulate N turnover (Cavalli & Bechini, 2011; Mohanty et al., 2011; Petersen et al., 2005b). Even if smaller RMSEs can be obtained by focussing on N dynamics separately (Cavalli & Bechini, 2012), considering multiple outputs ensured that the results of this research are useful for model applications aimed to simulate both C and N turnover. The adopted model analysis did not enable the contribution of model structure (model type) and parameters to total output uncertainty to be separated (Wallach et al., 2016). However, similar distributions of the simulated outputs among models (Figure 3) suggested that model-type had less effect on the simulated outputs than the parameters.

**Parameter distributions**

**Substrate use efficiency**

The range 0.27–0.87 included most of the Eff values adopted in mechanistic models similar to MOD1–4 (Henriksen et al., 2007; Noirot-Cosson et al., 2017; Whitmore, 2007). We assumed that Eff was constant over time, independent of N availability in soil, and was equal for all C mineralization fluxes (Figure 2). This approach is frequently adopted because it simplifies model initialization and the results obtained are satisfactory (Henriksen et al., 2007; Petersen et al., 2005b). Even if variable Eff among fluxes could represent some microbial features better (e.g. metabolic costs associated with the decomposition and assimilation pathways of molecules with different chemical structure) (Manzoni et al., 2012; Mooshammer et al., 2014; Sinsabaugh et al., 2013), it would require
the explicit representation of other microbial processes, including enzyme production, maintenance
and overflow metabolism that increase model complexity (Manzoni & Porporato, 2009; Sinsabaugh
et al., 2013).

**Microbial biomass C/N ratio**

The C/N of soil microbial biomass was constrained to the values reviewed for cropland, grassland,
pastures and bare soils by Xu et al. (2013). This key parameter contributes to positive or negative
NNM (mg N kg\(^{-1}\)) (values in each decomposition flux (NNM\(_{\text{flux}}\); Manzoni & Porporato, 2009):

\[
\text{NNM}_{\text{flux}} = K_S \times C_S \times \left(\frac{1}{C/N_S} - \frac{\text{Eff}}{C/N_B}\right),
\]

where subscripts S and B represent substrates and microbial biomass pools, respectively.

The ratio between C/N\(_B\) and Eff determines the substrate C/N threshold above which net N
immobilization occurs (Manzoni & Porporato, 2009). This threshold averaged 12 (Table 1), which is
slightly smaller than the range 15–35 estimated from incubation experiments involving soils amended
with manures, organic fertilizers and crop residues (Cavalli et al., 2016).

The variation of microbial C/N across different environments is narrow with a range of 4–10 g C g
N\(^{-1}\) (Xu et al., 2013) and contrasts with that of substrates, which cover values from <10 (e.g. young
leaves) to >100 (e.g. cereal straw) (Henriksen et al., 2007). This suggests that microorganisms are
mostly homoeostatic, even if adaptation of their C/N was reported as a strategy to overcome resource
imbalance (Mooshammer et al., 2014). This last approach was implemented in MOD1 and MOD3,
that allowed the C/N ratio of SMB (and of all native soil pools in MOD3) to vary temporarily as a
function of N availability.

**Partitioning of C and N in manure pools**

The sizes of manure pools MR and MVR were initialized following the reference distribution of
holocellulose and lignin in manures, respectively, while ML comprised a mixture of simple soluble
molecules (volatile fatty acids, simple sugars, amino acids, amino sugars). This approach was has
been used successfully used in previous research (Henriksen et al., 2007; Mohanty et al., 2011; Probert et al., 2005) and enabled the number of parameters to be calibrated to be reduced, which limited the risk of finding parameter sets that performed equally. Nevertheless, real turnover of SOM depends on other processes also (e.g. physical protection within soil macroaggregates, chemical sorption to clay minerals and Fe and Al oxides) and the status of the soil (e.g. temperature, moisture content) (Campbell & Paustian, 2015 and in Dungait et al., 2012). Therefore, chemically homogeneous model pools are expected to decompose heterogeneously in soil (Derrien & Amelung, 2011; Glanville et al., 2012). To overcome this limitation, alternative methods can be used to define manure pools kinetically. They assign C and N contents to model pools that optimize the simulation of experimental dynamics (Gillis & Price, 2016; Mondini et al., 2017; Noirot-Cosson et al., 2017; Petersen et al., 2005b), and assume implicitly that fast- and slow-decay pools are chemically heterogeneous mixtures of molecules.

Decay of manure pools

Most decay constants of manure pools were comparable to those reported for APSIM (Mohanty et al., 2011), a modified version of RothC (Mondini et al., 2017), MOTOR (implementing a model similar to RothC) (Whitmore, 2007), NCSOIL (Noirot-Cosson et al., 2017), CN-SIM (Cavalli & Bechini, 2011 and 2012; Petersen et al., 2005b) and the model of Henriksen et al. (2007). Indeed, MRT of ML varied from 4 to 9 days for crop residues and animal manures. The range for MR was wider, and the MRT values were as small as 20 days in APSIM and MOTOR, and as large as 1.1–2.7 years for the modified RothC (Mondini et al., 2017) and NCSOIL (Noirot-Cosson et al., 2017). Models also differed in the turnover of MVR: those similar to MOD4 (Henriksen et al., 2007; Petersen et al., 2005b) and MOD3 (Mondini et al.; 2017) adopted an MRT of 3–50 years, whereas studies using APSIM (Mohanty et al., 2011; Probert et al., 2005) used a smaller value (105 days), in accordance with the results of MOD2.
The MRT of manure pools sometimes deviated from incubation and field measurements that traced
the fate of $^{13}$C- and $^{14}$C-labelled substrates in soil (indicated by numbered boxes in Figure 4) including
low molecular weights compounds (average MRT = 21 days; Glanville et al., 2012), cellulose (MRT
in the range 0.9–1.6 years; Fioretto et al., 2005; Gunina & Kuzyakov, 2015) and lignin (MRT from
1.5 to 7.8 years; Derrien & Amelung, 2011; Fioretto et al., 2005).

**Sensitivity of model outputs**

Sensitivity analysis showed that only a few parameters effectively influenced net CO$_2$ emissions and
NNM (Figure 5 and 6; Figure S4 in Supporting Information). The most important parameters common
to all models were related to soil microbial biomass and to manure pools: the substrate use efficiency
(Eff), the C/N of microbial biomass ($C_{\text{SMB}}$ or $C_{\text{SMB2}}$), the fraction of manure C allocated to the
resistant pool ($F_{\text{MR}}$) and the manure decomposition constant $K_{\text{MAN}}$ for MOD1, or $K_{\text{ML}}$ for MOD2–
4. Such parameters should be adjusted when simulating specific soil • manure combinations. Ideally,
it would be reasonable that when different manures are applied to the same soil, a single set of
parameters related to soil and microbial biomass pools is shared among simulations, while parameters
describing manures are varied. The opposite can be postulated when the same manure is applied to
different soils.

However, sensitivity of both outputs to the variation in parameters linked to manure pools increased
as model complexity increased (from MOD1 to MOD4), especially in the medium term (after day
42). This result suggests that simple models like MOD1 necessitate the adjustment of all parameters
(both those related to soil, SMB and manure pools) for each manure type to obtain satisfactory
predictions, even when the same manure is applied on different soils. For example, the parameters
$K_{\text{MAN}}$, but also Eff and $C_{\text{SMB}}$, are set according to the manure C/N ratio in the STICS model (Brisson
et al., 2009; Giacomini et al., 2007). Conversely, more complex models that represent organic inputs
with multiple pools (MOD2–4) could differentiate soil or manure related parameters only, as required
by the specific conditions of the simulation (Petersen et al., 2005a and 2005b; Probert et al., 2005).
Results of sensitivity analysis depended on the amount of mineral N in soil (Figure 6b; Figure S4b in Supporting Information). Positive or negative NNM depended mainly on the combination of five parameters (those reported in Equation (2) plus the fractions of manure C and ON allocated to the manure pools). However, MOD3 reduced N limitation by temporarily increasing the C/N ratio of SMB, and for this reason the interactions among parameters were weak. Similarly, MOD1 allowed SMB to vary its C/N ratio temporarily according to microbial N requirement, but this strategy was not enough to balance the N deficit fully. For this reason, interactions in MOD1 were similar to MOD2 and MOD4, which had microbial pools with a fixed C/N ratio when N limitation became relevant (especially with manure C/ON ratio of 34). Fewer interactions among parameters could reduce calibration efforts of models like MOD1 and MOD3, characterized by variable C/N ratios in their pools, because the first-order effects of parameters are less influenced by soil mineral N concentration.

Results of sensitivity analysis depended on the statistical distribution of the parameters that we chose, the simulation scenarios (combinations of decomposition time, soil mineral N concentration and manure C/ON) and the model outputs considered (Pianosi et al., 2016). However, for calibration purposes, where the interest is in parameters ranking according to first-order indices, sensitivity analysis appears quite robust to alternative assumptions about the distributions of parameters, as suggested by previous analyses with the ICBM/2BN (similar to MOD2) and CN-SIM (same implementation as MOD4) models (Cavalli & Bechini, 2011; Cavalli et al., 2016).

**Limitations of the study and future developments**

Sensitivity analysis considered model outputs, net of the contribution of unamended soils, simulated over six months. This approach is valuable to study manure turnover because decomposition is mostly a fast process and, at the end of a six-month period, simulated C and N dynamics are often similar to those of unamended soils. Model applications including C and N released from native SOM would require longer simulation periods to fully appreciate the decomposition of soil pools with a slow
turnover. However, after defining additional parameter distributions to initialize soil native pools (Dungait et al., 2012; Falloon & Smith, 2000) the proposed methodology can be extended to the sensitivity analysis of gross outputs (i.e. CO$_2$ and mineral N from both manure and soil pools).

Considering that the mineralization of native SOM produces a relevant fraction of total CO$_2$ emitted from manure-amended soils (about 50%; Bechini & Marino, 2009), it is likely that the sensitivity of gross outputs to variation of parameters related to soil pools will be larger than that of net outputs to variations of the same parameters.
Conclusions

This study was the first in which the controls on C and N dynamics in modules of detailed structures (crop and grassland models) have been assessed and compared. The chosen modules derived from similar principles (discrete compartmental soil organic matter representation, first-order decomposition kinetic), but differed in the complexity of their regulation (number of parameters, pools and fluxes) of N and C turnover (fixed or variable C/N of model pools). The complexity of responses related to C respiration and N immobilization were taken into account in manure-amended soils with the support of incubation experiments. The study showed that all considered modelling approaches might provide a reasonable representation of the statistical distribution of soil fluxes (notably, net CO$_2$ emissions and net N mineralization) measured after the addition of manure. Furthermore, sensitivity analysis indicated that variation in the two output responses was mostly affected by a few influential parameters (microbial substrate use efficiency, C/N ratio of the soil microbial biomass, manure pools decomposition constants, partitioning factors of manure C among model pools).

The effect of parameters linked to manure pools increased with model complexity (in the order from MOD1 to MOD4), suggesting that calibration strategies could vary depending on the type of model. Models similar to MOD1 (with a single manure pool) probably require specific soil • manure parameterization, whereas more complex models (with multiple manure pools, like MOD2–4) could require only soil- or manure-specific calibrations, depending on model applications. The results of sensitivity analysis also showed that interactive effects among parameters tended to become more pronounced when C respiration rates deviated from potential rates because N limited soil organic matter decomposition. It appears that approaches similar to MOD1 and MOD3, implementing variable C/N ratios in their pools, were less sensitive to variation in soil mineral N availability and can be easily applied to a broad range of situations when properly calibrated.
This study has gone beyond the multi-model ensembles, which simulate crop and grassland systems in their integrated structure, but whose scope was not intended to be an exhaustive assessment of key processes. Although the modelling approaches we selected are only a subset of all available C–N modules, they represent a reasonable balance of contrasting approaches, which were implemented in several agricultural system models used worldwide. Our results will be useful for model improvements because modelling approaches are increasingly used to investigate changes in net terrestrial–atmosphere C–N exchanges, and thus evaluate mitigation options to reduce the emissions of greenhouse gases in crops and grasslands.

Supporting Information

Table S1. Main changes applied to the EPIC C–N module.

Table S2. Initialization of models during calibration and sensitivity analysis.

Table S3. List of papers used to define distributions of model parameters (Table 1) and to obtain measured data (Figure S2).

Table S4. Relative root mean squared errors between frequencies of measured and simulated net CO$_2$ emissions (mg C 100 mg manure C$^{-1}$) and net N mineralization (mg N 100 mg manure C$^{-1}$) before and after parameters calibration.

Figure S1. Schematic procedure used to calibrate parameters of models.

Figure S2. (a) Reference datasets of net CO2 emissions and net (b) N mineralization measured in laboratory incubation experiments from manure-amended soils. Continuous black line and symbols indicate the average of all treatments (labels indicate the number of data used to estimate means); dashed black lines indicate 5th and 95th percentiles; continuous grey lines indicate individual treatments (taken from the literature sources reported in Table S3); error bars indicate standard error of the mean.
Figure S3. (a) Uncertainty of net CO\(_2\) emissions (mg C 100 mg manure C\(^{-1}\)) and (b) net N mineralization (mg N 100 mg manure C\(^{-1}\)) prior to calibration of parameters. On the x-axis the central value of each class is reported.

Figure S4. (a) Sobol first-order sensitivity indices and (b) interaction terms for net CO\(_2\) emissions and net N mineralization calculated for eight dates over the period days 3–180. Results refer to a small initial soil mineral N concentration and manure C to organic N ratio of 34. Only model parameters with at least one first-order index or interaction term >10% are shown. The symbol ‘Σ’ indicates the sum of first-order sensitivity indices. See Figure 2 for names of the pools and Table 1 for a description of model parameters.

Acknowledgements

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Figure 1. Flowchart of model analysis by sensitivity analysis. Lower dashed box: traditional approach, consisting of running a sensitivity analysis prior to model calibration. Upper continuous box: work carried out in this study (see the text for details).

Figure 2. Structure of the four models. Rectangles represent C pools, valves represent C rates, arrows represent C flows and dot-ended arrows represent CO₂ emissions. Eff, microbial substrate use efficiency; FC, fraction of manure C allocated to labile (FC₉), resistant (FC₉), and very resistant (FC₉) manure pools. For a description of model parameters refer to Table 1.

Figure 3. (a) Uncertainty of net CO₂ emissions (mg C 100 mg manure C⁻¹) and (b) net N mineralization (mg N 100 mg manure C⁻¹) after calibration of parameters. On the x-axis the central value of each class is given.

Figure 4. Values of first-order decomposition constants for all pools of the four models. The values shown were obtained after multiplying the calibrated values by model-specific modification factors for the specific soil conditions. See Figure 2 for names of the pools. Areas with numbers 1–3 limit the interval of decomposition constants derived from MRT of low molecular weight compounds (1; Glanville et al., 2012), cellulose (2; Fioretto et al., 2005; Gunina & Kuzyakov, 2015) and lignin (3; Derrien & Amelung, 2011; Fioretto et al., 2005).

Figure 5. Sobol first-order sensitivity indices (% of total output variance) for net CO₂ emissions (mg C 100 mg manure C⁻¹) and net N mineralization (NNM; mg N 100 mg manure C⁻¹) calculated for eight dates over the period days 3–180. Results refer to large initial soil mineral N concentration and manure C to organic N ratio equal to 21. Only model parameters with at least one first-order index or interaction term larger than 10% are shown. The symbol ‘Σ’ indicates the sum of first-order sensitivity indices. See Figure 2 for names of the pools and Table 1 for a description of model parameters.

Figure 6. (a) Sobol first-order sensitivity indices and (b) interaction terms for net CO₂ emissions (mg C 100 mg manure C⁻¹) and net N mineralization (NNM; mg N 100 mg manure C⁻¹) calculated for eight dates over
the period days 3–180 with models MOD1–4. Results refer to small initial soil mineral N concentration and a
manure C to organic N ratio equal to 21. Only model parameters with at least one first-order index or interaction
term >10% are shown. The symbol ‘Σ’ indicates the sum of first-order sensitivity indices. See Figure 2 for
names of the pools and Table 1 for a description of model parameters.
a Review of manure decomposition in soil
b Review of soil and manure properties

Reference distributions for net CO₂ and net N mineralization  
Initial parameter distributions

Parameter calibration  
Revised parameter distributions

c Sensitivity analysis

Parameter ranking and selection

Experimental measures  
Parameter calibration  
To adapt model to a specific context
Abbreviations:
MAN, manure; ML, manure labile; MR, manure resistant; MVR, manure very resistant; SMB, soil microbial biomass; SMB1, autochthonous SMB; SMB2, zymogenous SMB; SMR, residues of SMB; HUM and NOM, active (native) stable SOM; SLOW, slow stable SOM; PASS, passive stable SOM; IOM, inert SOM
Figure 3

(a) Measured

Day 7 MOD1 MOD2 MOD3 MOD4

Day 14

Day 28

Day 42

Day 84

Day 180

(b) Measured

Day 7

Day 14

Day 28

Day 42

Day 84

Day 180
Figure 4

MOD1  MOD2  MOD3  MOD4

K$_{HUM}$  K$_{MVR}$  K$_{SMB}$  K$_{ML}$

First-order decomposition constant/day$^{-1}$

1.0E-6  1.0E-5  1.0E-4  1.0E-3  1.0E-2  1.0E-1  1.0E+0
Figure 5

MOD1  MOD2  MOD3  MOD4

% CO₂-C variance

% NNM variance

Time/days

Eff ▲ CNSMB/CNSMB2 ▲ CNSMB1 □ FCMR □ FCMVR ▲ FMVR ● KMAN/KML ○ KMR × ∑
Figure 6

(a)  

(b)  

% CO₂-C variance 

% NNM variance 

MOD1  MOD2  MOD3  MOD4  MOD1  MOD2  MOD3  MOD4 

Time/days  Time/days  Time/days  Time/days  Time/days  Time/days  Time/days  Time/days
Table 1. Parameters of the four models compared and their sampling distributions after calibration. For a description of C pools refer to Figure 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Name</th>
<th>References</th>
<th>Model MOD1</th>
<th>Model MOD2</th>
<th>Model MOD3</th>
<th>Model MOD4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters of soil microbial biomass pools</td>
<td></td>
<td></td>
<td>MOD1</td>
<td>MOD2</td>
<td>MOD3</td>
<td>MOD4</td>
</tr>
<tr>
<td>Substrate use efficiency / mg C mg C⁻¹</td>
<td>Eff</td>
<td>I</td>
<td>U(0.25–0.87)⁹</td>
<td>U(0.25–0.87)</td>
<td>U(0.25–0.87)</td>
<td>U(0.25–0.87)</td>
</tr>
<tr>
<td>C to N ratio / mg C mg N⁻¹</td>
<td>SMB</td>
<td>z</td>
<td>N(6.54,2.46)⁶</td>
<td>N(6.54,2.46)</td>
<td>N(6.54,2.46)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SMB1</td>
<td>Z</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>N(6.54,2.46)</td>
</tr>
<tr>
<td></td>
<td>SMB2</td>
<td>z</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>N(6.54,2.46)</td>
</tr>
<tr>
<td>First-order decomposition constant / day⁻¹</td>
<td>SMB</td>
<td>K_{SMB}</td>
<td>Optimized</td>
<td>N(2.79⋅10⁻²,4.85⋅10⁻⁵)</td>
<td>N(1.18⋅10⁻¹,8.75⋅10⁻⁴)</td>
<td>N(7.36⋅10⁻²,3.39⋅10⁻⁴)</td>
</tr>
<tr>
<td></td>
<td>SMB1 (total turnover)</td>
<td>T_{SMB1}</td>
<td>e,q</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SMB2 (total turnover)</td>
<td>T_{SMB2}</td>
<td>Optimized</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SMB1 (decay)</td>
<td>K_{SMB1}</td>
<td>q,r</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SMB2 (decay)</td>
<td>K_{SMB2}</td>
<td>q,r</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SMB1 (maintenance)</td>
<td>M_{SMB1}</td>
<td>q,r</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SMB1 (maintenance)</td>
<td>M_{SMB2}</td>
<td>q,r</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Turnover ratio of SMB1</td>
<td>R_{SMB1}</td>
<td>q,r</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Turnover ratio of SMB2</td>
<td>R_{SMB2}</td>
<td>r</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Parameters of native (&quot;humified&quot;) soil organic matter pools</td>
<td></td>
<td></td>
<td>MOD1</td>
<td>MOD2</td>
<td>MOD3</td>
<td>MOD4</td>
</tr>
<tr>
<td>C to N ratio / mg C mg N⁻¹</td>
<td>HUM</td>
<td>d,t</td>
<td>N(9.52,0.91)</td>
<td>N(6.00,0.36)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SLOW</td>
<td>k</td>
<td>–</td>
<td>–</td>
<td>N(12.00,1.44)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>PASS</td>
<td>k</td>
<td>–</td>
<td>–</td>
<td>N(7.00,0.49)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SMR</td>
<td>e,r</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>N(7.16,0.51)</td>
</tr>
<tr>
<td></td>
<td>NOM</td>
<td>q</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>N(10.00,1.00)</td>
</tr>
<tr>
<td>First-order decomposition constant / day⁻¹</td>
<td>HUM</td>
<td>K_{HUM}</td>
<td>d,t</td>
<td>N(3.48⋅10⁻⁴,7.58⋅10⁻⁹)</td>
<td>N(1.50⋅10⁻⁴,1.41⋅10⁻⁹)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SLOW</td>
<td>K_{SLOW}</td>
<td>k</td>
<td>–</td>
<td>–</td>
<td>N(5.00⋅10⁻⁴,1.56⋅10⁻⁴)</td>
</tr>
<tr>
<td></td>
<td>PASS</td>
<td>K_{PASS}</td>
<td>k</td>
<td>–</td>
<td>–</td>
<td>N(1.20⋅10⁻⁵,9.00⋅10⁻¹²)</td>
</tr>
<tr>
<td></td>
<td>SMR</td>
<td>K_{SMR}</td>
<td>e,r</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>NOM</td>
<td>K_{NOM}</td>
<td>q</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fraction of decomposed manure</td>
<td>H_{ManSmb}</td>
<td>t</td>
<td>–</td>
<td>U(8.10⋅10⁻¹,9.90⋅10⁻¹)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>directed to SMB / mg C mg C⁻¹</td>
<td></td>
<td></td>
<td>MOD1</td>
<td>MOD2</td>
<td>MOD3</td>
<td>MOD4</td>
</tr>
</tbody>
</table>

Footnotes:
- ⁹: U: uniform distribution
- ⁶: N: normal distribution
- ⁴: Calculated
- ³: Calculated

References:
- a: The parameter is not relevant for the given model.
- b: The parameter is constrained by the model's optimization.
- c: The parameter is a combination of two parameters, where the first parameter is unconstrained and the second is constrained by the model's optimization.
- d: The parameter is a calculated value.

Note: The values in parentheses represent the range or mean ± standard deviation, depending on the distribution type.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Name</th>
<th>References</th>
<th>Model</th>
<th>Model</th>
<th>Model</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction of recycled dead SMB / mg C mg C(^{-1})</td>
<td>(H_{\text{SMB}})</td>
<td>t</td>
<td>–</td>
<td>(U(5.40 \cdot 10^{-1},6.60 \cdot 10^{-1}))</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fraction of decomposed SMB directed to PASS / mg C mg C(^{-1})</td>
<td>(F_{\text{SMBPass}})</td>
<td>k</td>
<td>–</td>
<td>–</td>
<td>0.0094/Eff</td>
<td>–</td>
</tr>
<tr>
<td>Fraction of decomposed SLOW directed to PASS / mg C mg C(^{-1})</td>
<td>(F_{\text{SlowPass}})</td>
<td>k</td>
<td>–</td>
<td>–</td>
<td>0.0048/Eff</td>
<td>–</td>
</tr>
<tr>
<td>Fraction of decomposed manure directed to SMB1 / mg C mg C(^{-1})</td>
<td>(F_{\text{SMB1}})</td>
<td>q</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(N(3.16 \cdot 10^{-1},6.23 \cdot 10^{-3}))</td>
</tr>
</tbody>
</table>

Parameters of manure pools

\(\text{C to ON ratio / mg C mg ON}^{-1}\) | \(CN_{\text{MAN}}\) | a–c,f–j,m–p,s,u–y | \(N(21.00,40.96)\) | \(N(21.00,40.96)\) | \(N(21.00,40.96)\) | \(N(21.00,40.96)\) |

Manure C partitioning / mg C mg manure C\(^{-1}\)

<table>
<thead>
<tr>
<th>Model</th>
<th>(FC_{\text{ML}})</th>
<th>a–c,f–j,m–p,s,u–y</th>
<th>1–FM(R_{\text{MR}})–FMV(R_{\text{V}})</th>
<th>1–FM(R_{\text{MR}})–FMV(R_{\text{V}})</th>
<th>1–FM(R_{\text{MR}})–FMV(R_{\text{V}})</th>
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<td>ML</td>
<td>–</td>
<td>1–FM(R_{\text{MR}})–FMV(R_{\text{V}})</td>
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<td>1–FM(R_{\text{MR}})–FMV(R_{\text{V}})</td>
<td></td>
</tr>
<tr>
<td>MR</td>
<td>N(0.440,0.007)</td>
<td>N(0.440,0.007)</td>
<td>N(0.440,0.007)</td>
<td>N(0.440,0.007)</td>
<td></td>
</tr>
<tr>
<td>MVR</td>
<td>N(0.170,0.002)</td>
<td>N(0.170,0.002)</td>
<td>N(0.170,0.002)</td>
<td>N(0.170,0.002)</td>
<td></td>
</tr>
</tbody>
</table>

Manure ON partitioning / mg N mg manure ON\(^{-1}\)

<table>
<thead>
<tr>
<th>Model</th>
<th>(FON_{\text{ML}})</th>
<th>a–c,f–j,m–p,s,u–y</th>
<th>1–FM(R_{\text{MR}})–FMV(R_{\text{V}})</th>
<th>1–FM(R_{\text{MR}})–FMV(R_{\text{V}})</th>
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<tr>
<td>ML</td>
<td>–</td>
<td>1–FM(R_{\text{MR}})–FMV(R_{\text{V}})</td>
<td>1–FM(R_{\text{MR}})–FMV(R_{\text{V}})</td>
<td>1–FM(R_{\text{MR}})–FMV(R_{\text{V}})</td>
<td></td>
</tr>
<tr>
<td>MR</td>
<td>N(0.170,0.002)</td>
<td>N(0.170,0.002)</td>
<td>N(0.170,0.002)</td>
<td>N(0.170,0.002)</td>
<td></td>
</tr>
<tr>
<td>MVR</td>
<td>N(0.140,0.002)</td>
<td>N(0.140,0.002)</td>
<td>N(0.140,0.002)</td>
<td>N(0.140,0.002)</td>
<td></td>
</tr>
</tbody>
</table>

First-order decomposition constant / day\(^{-1}\)

<table>
<thead>
<tr>
<th>Model</th>
<th>(K_{\text{MAN}})</th>
<th>Optimized</th>
<th>(N(1.51 \cdot 10^{-2},1.43 \cdot 10^{-5}))</th>
<th>–</th>
<th>–</th>
<th>–</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML</td>
<td>(K_{\text{ML}})</td>
<td>Optimized</td>
<td>(N(5.65 \cdot 10^{-1},2.00 \cdot 10^{-2}))</td>
<td>(N(2.40 \cdot 10^{-1},3.59 \cdot 10^{-1}))</td>
<td>(N(7.66 \cdot 10^{-2},3.67 \cdot 10^{-4}))</td>
<td></td>
</tr>
<tr>
<td>MR</td>
<td>(K_{\text{MR}})</td>
<td>Optimized</td>
<td>(N(1.02 \cdot 10^{-2},6.45 \cdot 10^{-6}))</td>
<td>(N(1.28 \cdot 10^{-2},1.03 \cdot 10^{-5}))</td>
<td>(N(1.98 \cdot 10^{-3},2.44 \cdot 10^{-7}))</td>
<td></td>
</tr>
<tr>
<td>MVR</td>
<td>(K_{\text{MVR}})</td>
<td>Optimized</td>
<td>(N(1.02 \cdot 10^{-2},6.45 \cdot 10^{-6}))</td>
<td>(N(1.02 \cdot 10^{-2},6.45 \cdot 10^{-6}))</td>
<td>Equal to (K_{\text{MR}})</td>
<td>Equal to (K_{\text{NOM}})</td>
</tr>
</tbody>
</table>

References listed in Table S3 (Supporting Information): a, Althaus et al. (2013); b, Amon et al. (2007); c, Bechini & Marino (2009); d, Brisson et al. (2009); e, Cavalli & Becchini (2011); f, Cavalli et al. (2014; 2016; 2017); g, Chadwick et al. (2000); h, Fuccella et al. (2012); i, Kirchmann & Lundvall (1993); j, Kyvsgaard et al. (2000); k, Izaurralde et al. (2006); l, Manzoni et al. (2012); m, Marino et al., 2008; n, Morvan et al. (2006); o, Morvan & Nicolardot (2009); p, Peters & Jensen (2011); q, Petersen et al. (2005a); r, Petersen et al. (2005b); s, Powell et al. (2006); t, Probert et al.
(1998); u, Reeves III & Van Kessel (2002); v, Sørensen (1998); w, Sørensen & Fernández (2003); x, Sørensen et al. (2003); y, Triolo et al. (2011); z, Xu et al. (2013).

b Uniform distribution; lower and upper limits are indicated in parenthesis.

c Normal distribution; mean and variance are indicated in parenthesis.

d Calculated according to Petersen et al. (2005a and 2005b).
Table S1. Main changes applied to the EPIC C–N module.

<table>
<thead>
<tr>
<th>Model pool</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural litter pool (MR + MVR)</td>
<td>The pool was split into two pools, representing the lignin-like and cellulose-like fractions, respectively. As in the original module (Izaurralde et al., 2006), rate of turnover of both pools was affected by the percentage of the lignin-like fraction in ‘structural litter’ (MR + MVR; Figure 2).</td>
</tr>
<tr>
<td>Leached organic matter pool</td>
<td>The LEACH pool (Izaurralde et al., 2006), which estimates the fraction of C lost by leaching, was not used, given the closed system in which C and N fluxes are assessed in this study.</td>
</tr>
<tr>
<td>Microbial biomass (SMB), slow (SLOW) and passive (PASS) stable organic matter</td>
<td>Parameters (decomposition constants and partitioning factors) of the fluxes from microbial biomass to SLOW and PASS pools and CO₂, and from SLOW to PASS and CO₂ (Figure 2) were redefined according to original parameter values to obtain fluxes with a common structure.</td>
</tr>
</tbody>
</table>

Table S2. Initialization of models during calibration and sensitivity analysis.

<table>
<thead>
<tr>
<th>Parameter or variable</th>
<th>Value / unit</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil water content</td>
<td>-0.05 / MPa</td>
<td>No limitation to microbial activity. Soil water potential was used instead of soil water content because it is frequently the reference variable used by models to describe water availability to microbes.</td>
</tr>
<tr>
<td>Soil temperature</td>
<td>20 / °C</td>
<td>Average value from reviewed experiments.</td>
</tr>
<tr>
<td>Soil texture</td>
<td>Loam / –</td>
<td>To achieve an average effect of soil texture on SOM decomposition.</td>
</tr>
<tr>
<td>Manure C applied</td>
<td>1000 / mg C kg⁻¹ soil</td>
<td></td>
</tr>
<tr>
<td>All soil C and N pools</td>
<td>0 / mg C and N kg⁻¹ soil</td>
<td>Model results were net of unamended soil (manure was the only source of C and ON, according to the definition of net model outputs), and the occurrence of priming effects was excluded.</td>
</tr>
<tr>
<td>Soil mineral N</td>
<td>100 / mg N kg⁻¹ soil</td>
<td>If not differently specified, the addition of 100 mg N kg⁻¹ soil avoids the limiting effect of N shortage on C decomposition.</td>
</tr>
<tr>
<td>Time step</td>
<td>0.25 / days</td>
<td>–</td>
</tr>
</tbody>
</table>
Table S3. List of papers used to define distributions of model parameters (Table 1) and to obtain measured data (Figure S2).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cavalli, D., Corti, M., Baronchelli, D., Bechini, L. &amp; Marino Gallina, P. 2017. CO₂ emissions and mineral nitrogen dynamics following application to soil of undigested liquid cattle manure and digestates. <em>Geoderma</em>, 308, 26–35.</td>
<td>a,b</td>
</tr>
<tr>
<td>Kyvsgaard, P., Sørensen, P., Møller, E. &amp; Magid, J. 2000. Nitrogen mineralization from sheep faeces can be predicted from the apparent digestibility of the feed. <em>Nutrient Cycling in Agroecosystem</em>, 57, 207–214.</td>
<td>a,b</td>
</tr>
</tbody>
</table>


“Papers used to define distributions of model parameters (Table 1) are identified by ‘a’; papers that were used to obtain net CO₂ and net N mineralization measurements (Figure S2) are identified by ‘b’. Some papers were used for both purposes (a,b).
Table S4. Relative root mean squared errors between frequencies of measured and simulated net CO$_2$ emissions (mg C 100 mg manure C$^{-1}$) and net N mineralization (mg N 100 mg manure C$^{-1}$) prior and after parameters calibration.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day</th>
<th>Errors prior calibration</th>
<th>Errors after calibration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOD1</td>
<td>MOD2</td>
<td>MOD3</td>
</tr>
<tr>
<td>Net CO$_2$ Emissions</td>
<td>3</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Net N Mineralization</td>
<td>3</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>26</td>
<td>22</td>
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<tr>
<td></td>
<td>42</td>
<td>25</td>
<td>24</td>
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<td></td>
<td>84</td>
<td>28</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>36</td>
<td>43</td>
</tr>
</tbody>
</table>
Main procedure (OMNIOPT genetic algorithm)
Initial parent population \( P \):
- create \( n \) (48÷80) individuals, each with a vector \( x \) of size \( d \) (=number of \( \mu \), 2–4).
- initialize \( x \) according to Latin hypercube sampling.
- divide \( P \) into \( s \) (=16) groups of \( v \) individuals (\( v=d+1 \) and \( n=s \times v \)).
  - Call Procedure 1.
- update \( P \) and calculate fitness (rank-based).

At each generation \( g \) (1–4):
- create an offspring population \( C \) of size \( n \) from \( P \).
- divide \( C \) into \( s \) groups of \( v \) individuals.
  - Call Procedure 1.
- update \( C \) and create a mixed population \( M=P+C \).
- calculate fitness and select best \( n \) individuals as new \( P \).

Procedure 1 (Nelder-Mead algorithm)
Initial \( s \) (=16) simplexes:
- for each simplex, create \( v \) vertexes, each with a vector \( x \) of size \( d \).
- initialize \( x \) with values provided by the genetic algorithm.
- evaluate vertexes of each simplex (call Procedure 2).
  - At each iteration \( i \) (1–15):
    - evaluate new vertexes (call Procedure 2).
    - stop if simplex collapsed or maximum number of iteration was reached.

Procedure 2 (Objective function evaluation)
At each function evaluation:
- create 4 vectors \( x, y, l \) and \( u \) of size \( d \) for distributions’ parameters \( \mu, \sigma (=0.25\mu) \), lower and upper limits (=\( \mu \pm 3\sigma \)), respectively.
- estimate simulated net CO\(_2\) and NNM uncertainty at each date (call Procedure 3).
- calculate errors (RMSE) between simulated and observed frequencies of net CO\(_2\) and NNM for the 8 dates.
- the function value is the average of all RMSEs.

Procedure 3 (Model outputs uncertainty)
For each iteration \( j \) (1–2048):
- initialize a vector \( z \) of size \( d \) with values uniformly distributed in the range 0–1 (Sobol’ sequence generator).
- initialize a vector \( z' \) of model parameters normally distributed (\( \mu, \sigma \)) using inverse cdf.
- run the model with generated parameters \( z' \).

Figure S1. Schematic procedure used to calibrate parameters of models.
Figure S2. (a) Reference datasets of net CO$_2$ emissions and net (b) N mineralization measured in laboratory incubation experiments from manure-amended soils. Continuous black line and symbols indicate the average of all treatments (labels indicate the number of data used to estimate means); dashed black lines indicate 5th and 95th percentiles; continuous grey lines indicate individual treatments (taken from the literature sources reported in Table S3); error bars indicate standard error of the mean.
Figure S3. (a) Uncertainty of net CO$_2$ emissions (mg C 100 mg manure C$^{-1}$) and net (b) N mineralization (mg N 100 mg manure C$^{-1}$) prior calibration of parameters. On the x-axis the central value of each class is reported.
(a)  

![Graph showing the percentage CO\textsubscript{2}C variance and NNM variance over time for MOD1, MOD2, MOD3, and MOD4.](image)

(b)
Figure S4. Sobol first-order sensitivity indices and (b) interaction terms for net CO$_2$ emissions (mg C 100 mg manure C$^{-1}$) and net N mineralization (NNM; mg N 100 mg manure C$^{-1}$) calculated for eight dates over the period days 3–180 with models MOD1–4. Results refer to small initial soil mineral N concentration and manure C to organic N ratio equal to 34. Only model parameters with at least one first-order index or interaction term $>$ 10% are shown. The symbol $\Sigma$ indicates the sum of first-order sensitivity indices. See Figure 2 for names of the pools and Table 1 for a description of model parameters.