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ABSTRACT

Crop diversification is a potentially attractive agricultural practice for enhancing organic carbon (C) storage in soils. The aim of the EJP Soil project EnergyLink is to better understand the link between crop diversity and carbon sequestration by the soil microbiome across a pan-European pedo-climatic gradient. The central hypothesis is that greater crop diversity results in more efficient microbial use of C, thus enhancing the potential of soils to store C. Work package 6 on "Soil Organic Matter Modelling" aims to assess the relative impact of including the soil microbiome in SOC-models. This report describes the elaboration of task 6.3, in which the selected models have be adapted to include the soil microbiome and subsequently run with data from LTEs participating in EnergyLink.

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Table of Contents

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 862695

PART 1 List of Figures, tables and acronyms and abbreviations

List of Figures

List of Tables

List of Acronyms and abbreviations

BAU	Business As Usual
BIO	Soil biological pool
C	Carbon
CCC	Clever Cover Cropping
Cmic	Microbial-C
CUE	Carbon Use Efficiency
DPM	Decomposable Plant Material
F	Fallow
GLU	Glucosidase hydrolysation
HC	Humification Coefficient
HUM	Humified Material
HWC	Hot Water-extractable Carbon
IOM	Inert organic Material
LAP	Leucine Aminopeptidase
MAOM	Mineral Associated Organic Matter
LTE	Long-Term Experiment
NPOC	N in Non-Particulate Organic C
O	Oat
OR	Oat, Radish
POM	Particulate Organic Matter
R	Radish
RMF	Rate Modifying Factor
ROI	Return On Investment
RPM	Resistant Plant Material
SOM/SOC	Soil Organic Matter resp. Carbon
V	Vetch
VO	Vetch, Oat
VOR	Vetch, Oats, Radish

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PART 2 List of Figures, tables and acronyms and abbreviations

List of Figures

List of Tables

List of Acronyms and abbreviations

AGG	Aggregate C
C	Carbon
CO_2	Carbon dioxide
MAOM	Mineral-associated organic matter
MIC	Microbial biomass C
LMWC	Low molecular weight carbon
POM	Particulate organic matter
SOC	Soil organic carbon

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1. Introduction

The aim of the EJP Soil project EnergyLink is to better understand the link between crop diversity and carbon sequestration by the soil microbiome across a pan-European pedo-climatic gradient of long-term field experiments (LTEs). Work package 6 on "Soil Organic Matter Modelling" aims to assess the relative impact of including the soil microbiome in SOC-models. In task 6.1, a hypothetical Energy-model (micro-level) for the relationship between the microbial biomass pool and SOC dynamics was discussed and potential indicators, i.e. microbial carbon use efficiency (CUE) and the energetic return on metabolic investment (ROI) screened for their suitability. Of these, CUE offered the best perspective for inclusion in SOC-models at both micro- and macro-level (Meurer et al., 2023). Task 6.2 aimed to screen current and next generation SOM (macro-) models for their suitability and adaptability to include the soil biome. The ROTHC and Millennial models were selected to explore the perspectives for inclusion of the soil microbiome, the former because it is much used at European scale-level, and the latter because it defines SOC-pools as measurable entities (Di Lonardo, 2023). We use the following definition for microbiome: "Microbiome is considered the diversity of different microbes (e.g. bacteria, fungi) and its theatre of activity" (Berg et al., 2020).

Objective
The present study aims to adapt and apply these models, evaluating the wide selection of measured soil microbiome data within EnergyLink for use in the modelling of the participating LTEs.

PART 1.

2. Modelling with ROTHC

2.1 Materials & Methods

2.1.1 Description of the model and modelling strategy

Description of the model
The original ROTHC model (vs. 26.3, (Coleman & Jenkinson, 1996, 2014) recognises 5 SOC pools of which the BIO pool represents carbon stored in the living microbial biomass. In the model, soil organic carbon is split into four active compartments and a small amount of inert organic matter (IOM). The four active compartments are Decomposable Plant Material (DPM), Resistant Plant Material (RPM), Microbial Biomass (BIO) and Humified Organic Matter (HUM). Each compartment decomposes by a first-order process with its own characteristic rate. The IOM compartment is resistant to decomposition. The structure of the decomposition process as included in the model is shown in Figure 1.

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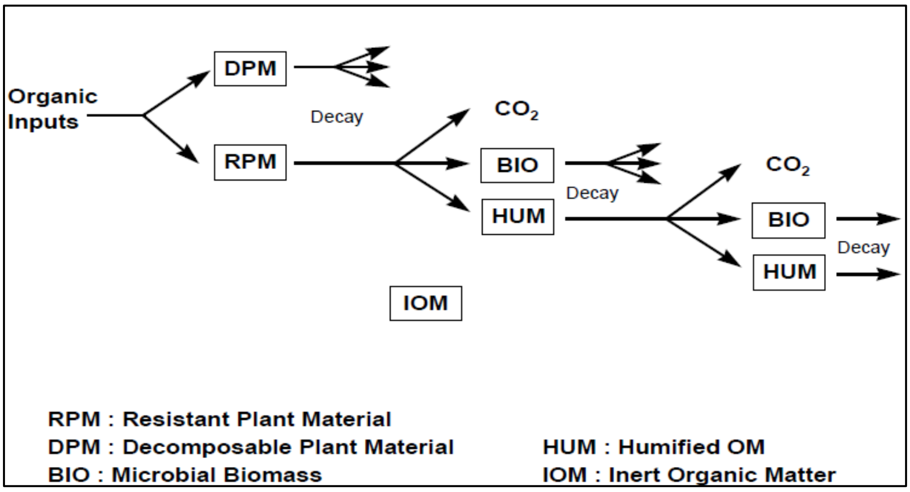

Figure 1. Schematic representation of the decomposition process in the Roth-C model (Coleman & Jenkinson, 2014).

In each pool, SOC is decomposed following the 1st order exponential function:

$Y_{t+1} = Y_t e^{-abckt}$, in which Eq. 1

Y_{t+1} = the amount of SOC at the end of the month
Y_t = the amount of SOC at *the beginning of the month*
a = the rate modifying factor for temperature
b = the rate modifying factor for moisture
c = the soil cover rate modifying factor
k is the decomposition rate constant for a pool
t is 1 / 12, since *k* is based on a yearly decomposition rate

To run the model, an initialization step is required to parameterize the model to local conditions. This is done by running the model with local data until equilibrium in SOC- contents is reached, which may be a period of 10.000 – 50.000 years. Subsequently, scenario analyses may be performed, using monthly and/or yearly input data of organic matter from crop and manure (Table 1).

Table 1. Required input data for the Roth-C model.

Input category	**Data required**
Weather	· Monthly rainfall (mm) · Monthly potential evapotranspiration (mm) · Average monthly mean air temperature (°C)
Soil	· Clay content of the soil (%) · Depth of soil layer sampled (cm)

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	·	Soil cover (yes / no)
Crop residues	·	Monthly input of plant residues (t C ha^{-1})
	·	An estimate of the decomposability of the incoming plant material, the DPM/RPM ratio
Farmyard manure	·	Monthly input of farmyard manure (t C ha^{-1})

The plant residue input is the amount of C that is put into the soil per month (t C ha^{-1}). The decomposability of crop residues and input from farmyard manure is characterized by the DPM/RPM ratio of the materials. For agricultural purposes, a value of 1.44 to the DPM/RPM ratio of crop residues may be used (Coleman & Jenkinson, 1996), with 59% of the plant material as DPM and 41% as RPM. The amount of farmyard manure (t C ha^{-1}) is treated differently from inputs of fresh plant residues as it is assumed to be already more decomposed than normal crop plant material. It is split in DPM 49%, RPM 49% and HUM 2%.

Both DPM and RPM decompose to form CO_2, BIO and HUM, with one proportion going directly to CO_2 and the other proportion to BIO + HUM. This partitioning is determined by the clay content of the soil. The BIO + HUM is then split into 46% BIO and 54% HUM. BIO and HUM both decompose to form more CO_2, BIO and HUM. As with other constants and functions within ROTHC, the partitioning is parameterised using data from a Rothamsted soil.

Modelling strategy
We used the RothC model script version in R (Sierra et al., 2012). To allow for differences in decomposability between cover crops, we used an adaptation for diversified C-input from (cover) based the linear relationship between the humification coefficient (HC) and the DPM/RPM-ratio (Gent University, 2008):

DPM/RPM = -2.174 HC + 2.020 (for HC ≤ 0.92)
DPM/RPM = 0 (for HC > 0.92) Eq. 2

The main objective of this research is to assess the impact of the soil microbiome on SOC-stock. The working hypothesis is that the soil microbiome exerts an impact on SOC stock as modelled with ROTHC-model. This impact could take effect in multiple ways, e.g., diversity, C biomass, activity, etc. It is therefore important to explore and test a variety of soil microbiome indicators. From a theoretical point of view, there are several options for improvement of how the soil microbiome is included in the model: change the BIO pool size, add another pool with different features (e.g., a fast and slow decomposing pool), change the partitioning over BIO and HUM, and/or add a RMF for the soil microbiome.

Preliminary calculations were made for these option using data from the literature for an arable rotation on sandy soil in The Netherlands. These explorations showed that of these options, probably only a RMF could have a distinct impact on the outcome of the modelling. Therefore, adding a RMF for the soil microbiome (RMF_{BIO}) was selected for a first assessment of the impact of the soil microbiome on SOC stocks. This RMF is to be allocated next to the other RMFs in the model (see Eq.

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1). In the exponential equation, RMFs are treated multiplicatively and so would an RMF_{BIO} be. By definition, a RMF of 1 corresponds to the model 'Business As Usual' (BAU).

To explore the possible use and impact of a modifying factor for the soil microbiome (RMF_{BIO}) on the performance of the model, comparison was made of the model 'as is' (BAU, business as usual) and the adapted model. The modelling results of both are compared to measured SOC-values during the experimental period by computing root mean squared errors (RMSE) between modelled and measured values. A series of RMFs from 0.1 to 3.0 with steps 0.1 was used to improve the fit. The optimal RMF was defined as the RMF with minimal RMSE for that treatment.

Subsequently, correlations analysis was performed between the optimal RMF and soil indicators including the microbiome. If substantial and significant correlations are found, than the RMF may considered to be, at least partially, related to the soil microbiome and the subscript 'BIO' is added to the RMF. The threshold for a substantial correlation was set at 0.4 (abs). When testing a high number of variables as in this case for the microbiome, with every single test the chance of incorrectly 'proving' significance increases. Therefore, a statistical correction (Bonferroni) is required that takes the number of tests into account. In this research, a p-value of 0.001 would be a good threshold.

Finally, the impact of the soil microbiome on SOC-dynamics was assessed as the difference between the models BAU and with RMF_{BIO}. This was done by modelling over the a period with known weather data , i.e., 2000 – 2020, to avoid the effect of uncertainties in future climate data on the outcomes.

2.1.2 Selection and description of LTE

Selection of LTEs
EnergyLink includes a North-South gradient of LTEs, in which crop diversity is a treatment. In these, extensive soil sampling has taken place for measurement of the soil microbiome. Subsequent data-collection aimed at basic soil features (clay content, bulk density, time series of SOC) and C-input from crops, in particular cover crops, as these are required for running ROTHC. From the LTE's participating in EnergyLink, only LTE Clever Cover Cropping in Wageningen, The Netherlands, provided all the required data. The ROTHC-modelling is therefore limited to this LTE.

LTE Clever Cover Cropping
The experiment Clever Cover Cropping focuses on the use of cover crops during the winter period in arable cropping systems. The field experiment is conducted on a sandy soil (Haplic podzol, 83% sand, 12% silt, 2% clay, pH 5.3, 1.09 g kg^{-1} N, 58 $mg.kg^{-1}$ P of which 2.2 mg,kg^{-1} plant-available P, and 91 $mg.kg^{-1}$ K) at the Nergena Farm, Wageningen, The Netherlands, 51°59'42.7''N, 5°39'35.7''E) (Elhakeem et al., 202023). The long term annual average temperature is 9.4°C and annual average rainfall is 780 mm. Drainage pipes are installed 100 cm deep. The field has a history of conventional arable farming, winterwheat being the last commercially crop grown before the start of the experiment in 2016. The experiment consists of eight cover crop treatments (Porre, 2020) : 3

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research and innovation programme under grant agreement N° 862695

monoculture species, Radish (Raphanus sativus, (R)), Vetch (Vicia sativa, (V)), and Oats (Avena strigoza, (O)) 3 mixtures of 2 species (RV, RO, VO) and 1 mixture of the 3 species (VOR), and a fallow control (F). The treatments are replicated five times in a complete randomized block design in plots of 60 m2 (10x6 m).

Input data and initialisation
At the start of the experiment in 2016, basic soil parameters were measured, e.g. clay-content. SOM data (loss on ignition) were available at plot level over the period 2016 - 2022 from which SOC was calculated using a factor of 0.58. In 2022, bulk density was measured at treatment level and assumed constant over the experimental period. For both main crops and cover crops, default data were used for C-input and humification coefficients were used. For all C-inputs, the DPM/RPM–ratio of C-input was assessed using equation Eq.1. Weather data were taken from the Royal Netherlands Meteorological Institute (KNMI). The model was set up with a monthly time-step. Initial SOC pool sizes were determined at treatment level using the PTFs by Weihermüller et al. (2013). Data for mean monthly potential evapotranspiration were taken from Müller (1982).

2.1.3 Soil analysis

Soil samples were taken in August 2022 in the layer 0-20 cm and in December 2022 in the layer 0-30 cm, and analysed for a wide range of over 30 indicators by various laboratories of partners within EnergyLink (See Annex 1 for the indicators and their abbreviations). Most indicators included in the correlation analysis are directly related to the soil microbiome while others relate to qualities of organic matter. No discrimination is made beforehand. All data are available at the EnergyLink repository.

2.2 Results

Assessment of optimal Rate Modifying Factor
The ROTHC-model was able to describe the time series of SOC-data reasonably well, as shown for VOR (Figure 2). Black dots denote the average of the measured SOC-values and their error bars. Coloured lines represent modelled results using a series of RMFs as correction factors. By definition, modelled SOC% decreases with the RMF. For VOR, the best fit between modelled and measure SOC was obtained for an RMF of 1.5 (dimensionless). RMSE was optimized by hand, which gave for all treatments the correct value for the optimal RMF with 0.1 precision.

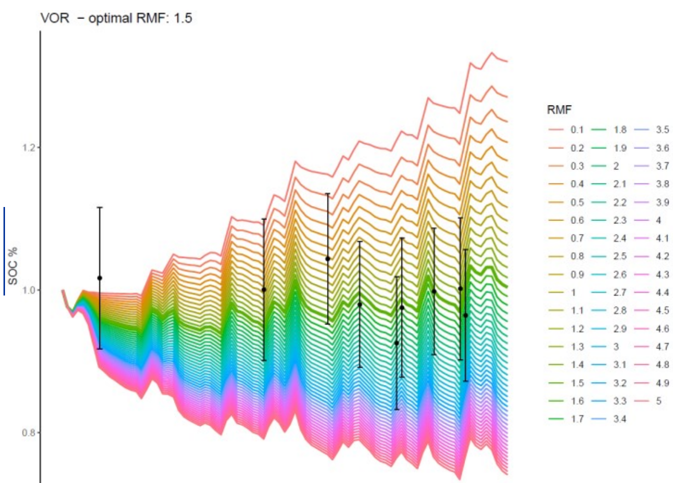

Figure 2. Evaluation of RMFs in ROTHC, treatment VOR.

Results for the other treatments are similar to VOR, except for the fallow (Annex 2). In the plots it can be seen (except the fallow) that starting from 0.1, first RMSE decreases, then reaches some optimal value and then increases again. Note that for RMF<1, SOC% is higher than in BAU, and for RMF > 1, SOC% is lower than in BAU. Over all cover treatments, optimal RMF ranged from 0.1 for the Fallow to 1.9 for Oats, respectively (Table 2; Annex 2). The fallow showed a poor fit which may be may be ascribed to the weeds that were growing proliferously (green fallow). Therefore, the fallow treatment is not further discussed.

Table 2. Optimal RMF for cover crops in LTE Clever Cover Cropping.

Cover Crop Treatment	RMF
VOR	1.5
O	1.9
VO	1.4
R	1.6
F	0.1
V	0.9
VR	1.4
OR	1.4

Exploration of the relationship between optimal RMF and indicators soil microbiome

In the exploration of indicators of the soil microbiome, priority was given to the Carbon Use Efficiency (CUE) as this indicator would be directly related to SOC storage and had been identified as promising (Meurer et al., 2023). Visual presentation of the data suggested some patterning between CUE and optimal RMF (Figure 3) with a correlation value of -0.33 (p<0.1).

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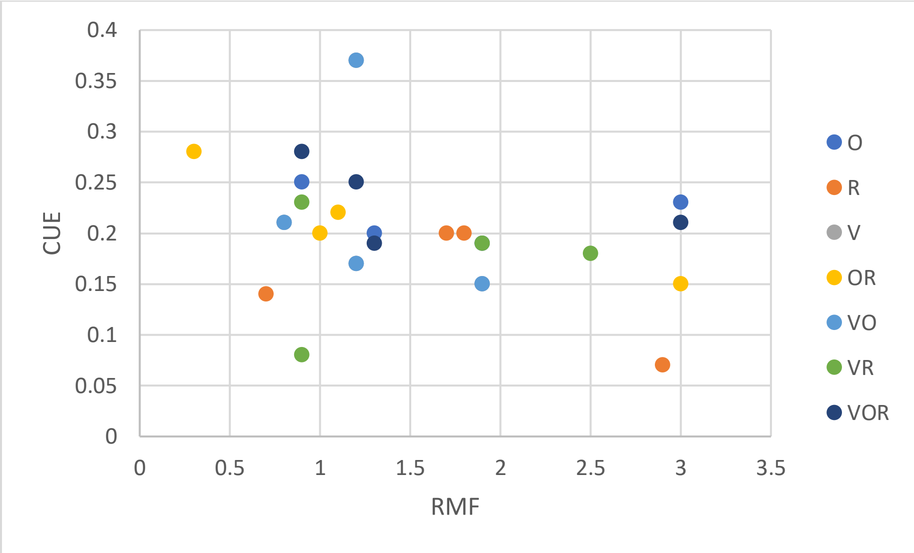

Figure 3. Scatterplot of RMF and CUE at treatment level.

In the overall correlation analysis between RMF and indicators for the soil microbiome, few indicators were found to have a substantial correlation factor (Table 3). Of these, the indicators LAP, Galactosamine, Glucosamine and Cmic are directly related to the soil microbiome; NPOC, POM, MAOM, HWC are related to the quality of soil organic matter. Note that regarding the indicators in Table 3, only NPOC is positively correlated.

Table 3. Indicators with high correlation- and p-values with the optimal RMF.

Indicator	Layer 0-20 cm	p-value	Layer 0-30 cm	p-value
LAP	-0.66	≤0.001	-0.64	≤0.001
GLU			-0.59	≤0.001
Galactosamine	-0.48	≤0.01		
Glucosamine	-0.44	≤0.1	-0.42	≤0.1
NPOC	0.44	≤0.1		
Cmic			-0.43	≤0.1
POM			-0.44	≤0.1
MAOM	-0.41		-0.71	≤0.1
HWC			-0.49	≤0.01

The significance of the indicators for LAP and GLU met the threshold, thus suggesting that the optimal RMF might be explained at least partially by the soil microbiome and the suffix BIO may be applied (RMF_{BIO}). A next step would be to formulate the relationships for LAP and GLU mathematically (Figure 4) in order to include as RMF_{BIO} in the ROTHC model.

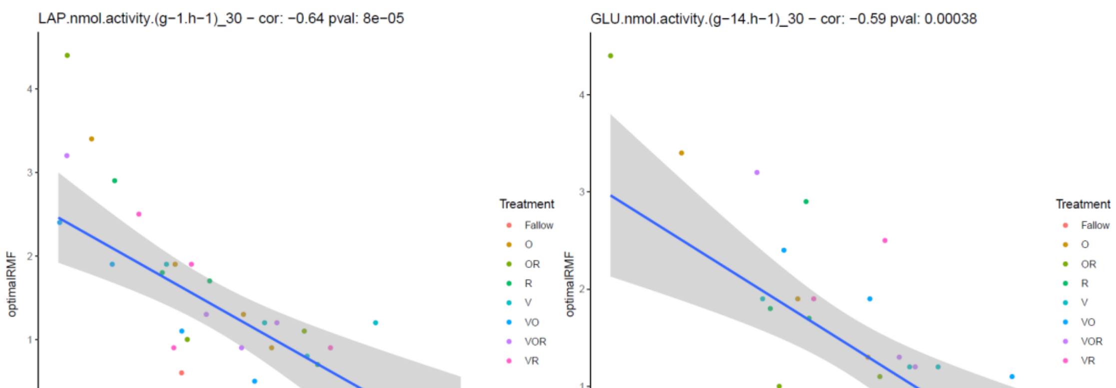

Figure 4. Relationships ($p<0.0001$) between RMF and indicators for the soil microbiome. Left: LAP (0-30 cm layer); right: GLU (0-30 cm layer).

It was found that for both LAP and GLU some of the measured values correspond to RMF > 1, indicating that net SOC accumulation may be lower than BAU.

Impact assessment

Over the period 2000 – 2020, the effect of taking into account RMF_{BIO} in the ROTHC model on SOC-stock differs per cover crop (Table 1). For all cover crop mixtures, SOC stocks are higher with BAU than with RMF_{BIO}. The impact on SOC stocks varies from -2.1 t ha^{-1} for Vetch to -5.8 t ha^{-1} for Oat.

Table 4. Modelled effect of the soil microbiome on SOC stock layer (0-20 cm) over 21 years.

Treatments	SOC%		SOC stock (t ha^{-1})		
	BAU	RMF_{BIO}	BAU	RMF_{BIO}	Difference
VOR	1.06	0.91	32	27	4.5
O	1.07	0.88	32	26	5.8
VO	1.12	0.99	33	29	3.8
R	1.12	0.94	34	28	5.3
V	1.13	1.06	34	31	2.1
VR	1.13	0.99	33	29	3.9
OR	1.10	1.00	32	29	2.9

2.3 Discussion

Impact

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We found that that the effects of cover crop diversity, resulting from the combination of C-input, HC, and winter cover, on SOC-dynamics over the experimental period are relatively small. There are several possible compelling reasons explaining why crop diversity may not always result in a positive impact SOC. First, while crop diversity is often assumed to enhance carbon inputs, the specific functional traits of the plant species involved are crucial. Not all crops contribute equally to SOC, particularly in terms of root biomass, residue quality, or carbon stabilization potential. Some species may produce lower quantities of biomass or decompose rapidly, which can limit the amount of carbon that is retained in the soil over time. Second, the timing and synchrony of carbon inputs could be mismatched in diverse systems. If different crops grow at different rates or deposit organic matter at varying times, it can lead to fluctuations in microbial activity and nutrient cycling, reducing the efficiency of carbon storage. In some cases, faster-decomposing crops can stimulate microbial respiration, leading to net carbon losses despite higher plant diversity. Third, management practices in diverse systems may inadvertently counteract the potential benefits of diversity. For instance, more diverse systems often require more intensive management, such as increased tillage or chemical inputs, which can disturb soil structure and enhance carbon mineralization, thereby reducing the overall carbon sequestration potential. Fourth, competition between crop species in a diverse system can reduce overall productivity. If crops compete for water, light, or nutrients, the total biomass produced—and thus the amount of organic carbon returned to the soil—may be lower than in monocultures or simple rotations with more complementary species. This competition can especially limit root biomass, which is a key contributor to long-term SOC storage. Lastly, environmental and soil-specific factors may modulate the effect of crop diversity. In some soils, particularly those with low organic matter content or poor structure, the potential benefits of diversity may be constrained by the soil's limited capacity to stabilize carbon. Similarly, in arid or nutrient-poor environments, the stress on crops can limit growth, biomass production, and carbon inputs, regardless of diversity.

In this study, the role soil microbiome in SOC dynamics was modeled using RMF_{BIO} parameter within the ROTHC framework. Table 4 highlights that the difference in SOC between BAU and the use of an optimal RMF ranges from -2.1 and -5.7 t ha^{-1} over 20 years, depending on the cover crop. This represents an decrease of 7 to 18 percent, assuming a SOC stock of 30 t ha^{-1}. While this difference may not seem extreme, it is still quite substantial. A significant proportion of this effect, attributed to the optimal RMF, can be explained by LAP and/or GLU, the top two variables in Table 3. Both LAP and GLU are indicators of extracellular enzyme activity (Inselsbacher et al., 2024) involved in mineralisation and/or stabilisation processes of organic matter. The observations suggests that incorporating RMF values specifically for LAP and GLU into the ROTHC model could yield results similar to those achieved with optimal RMF. Note that Table 3 shows for both indicators, that at plot level in LTE Clever Cover cropping, RMF may occur that are lower and/or higher than 1. This would imply that SOC accumulation may be higher and lower, respectively, as compared to BAU. However, further research is needed to confirm this, as the current findings are based on data from a single LTE.

A key observation from the study is that crop diversity did not have a direct, positive effect on SOC stock. In BAU, the range in SOC stock was 32 – 34 t ha^{-1}, with highest stock shown for radish and vetch

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in monocultures. With the RMF_{BIO}, range was 26 – 31 t ha^{-1}, for oats and vetch, respectively. The differences between the effects of oats and vetch suggest that N-fixing cover crops like vetch may interact more with the soil microbiome compared to cereals, such as oats. Nitrogen provided by vetch may stimulate microbial activity, accelerating the turnover of organic matter, which, for LTE Clever Cover Cropping, resulted in higher SOC stock as compared to the other cover crop mixtures.

Moreover, the findings suggest that that crop diversity could have a “levelling” effect on decomposition rates by balancing C and N inputs from different species. This balance might moderate the rate at which organic matter is broken down, preventing the accumulation of SOC. Additionally, the quality of the plant residues—specifically their carbon-to-nitrogen ratio—could influence the partitioning of organic material into different soil carbon pools, such DPM and RPM, or the distribution between BIO and humified organic HUM. However, the precise effects of substrate quality on these SOC fractions have not been fully explored in this study, leaving room for further investigation. Overall, these findings emphasize that while crop diversity can influence soil processes, its impact on SOC accumulation is complex and highly dependent on species traits and their interactions with the soil microbial community.

Further research

The RMF, assessed by optimization, does not exclusively refer to the soil microbiome, as it may also account for measurement errors. As it is known that temperature exerts an effect on the soil microbiome, it can be inferred that the RMF for temperature already accounts to some extent for the soil microbiome. Future research is needed to understand why indicators would have a direct relationship with SOC-dynamics, and if they are mutually exclusive or additive etc., which was beyond the scope of this study.

Among the indicators tested, some directly reflect soil microbiome activity, while others relate more closely to the quality of the SOC-pools. The shortlist of indicators likely has specific relevance to the LTE Clever Cover Cropping. Previous research (Porre, 2020) suggested that this LTE is nearing SOC saturation, raising questions about the applicability of the shortlisted indicators during early SOC build-up phases. During initial SOC-built-up microbial contributions may play a more pivotal role than SOC pools themselves, potentially shifting the relevance of these indicators.

As the model itself was developed c. half a century ago, one could argue that the ROTHC concept falls short in taking into account the latest scientific knowledge on SOC-dynamics, e.g., soil biodiversity, roots, dead microbial biomass, and quality of the carbon input. However, over time the available evidence has proven the model to be applicable in cropping systems in a variety of soil and climate conditions. From a modeling perspective, only a limited number of parameters are necessary to evaluate SOC-dynamics. However, to facilitate further analysis of a diversity in C-input and /or the soil microbiome, it is crucial to collect baseline soil data—such as SOC content, clay percentage, and bulk density—at the plot level. This foundational information would allow for more refined analyses of how the soil microbiome and SOC pools interact over time, particularly in different stages of SOC build-up.

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2.4 Conclusion

Modeling SOC dynamics in an arable rotation with cover crops on sandy soil in NW Europe revealed that including the soil microbiome, or certain microbial parameters, has potential to improve simple carbon balance models. The unique approach taken here should be tested in different situations to confirm that extracellular enzyme activity is particularly valuable to further constrain the RothC model.

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Annex 1. List of indicators checked for correlation.

Indicator		Indicator	
Name	Abbreviation and/or Unit	Name	Abbreviation and/or Unit
Carbon Use Efficiency	CUE	Galactosamine/Glucosamine	Gal/Gluc
Leucine Aminopeptidase	LAP nmol activity (g^{-1} h^{-1})	Monnasamine	(µg/gDW)
ß-N-acetyl glucosaminidase	NAG nmol activity (g-1 h-1)	Muramic acid	(µg/gDW)
Glucosidase hydrolysation	GLU nmol activity (g-14 h-1)	Galactosamine	(µg/gDW)
Phosphatase Enzyme activity	PHO nmol activity (g-1 h-1)	Glucosamine	(µg/gDW)
C acquisition rate per unit microbial biomass carbon	Caqu	Total Dissolved Arsenic	AStot (µg/gDW)
N acquisition rate per unit microbial biomass carbon	Naqu	Microbial C	mic C [µgC/gDW]
P acquisition rate per unit microbial biomass carbon	Paqu	Microbial N	mic N [µgN/gDW]
Total enzymatic activity per unit microbial biomass carbon	EEA/micC	Microbial P	mic P [µgP/gDW]
Fungal Growth Rate	(µgC gDW-1 h-1)	Microbial C to N ratio	C:N ratio mic
Bacterial Growth Rate	(µgC gDW-1 h-1)	N in Non-Particulate Organic C	NPOC (mg(gDW)
Microbial Turn Over	(days)	Total Dissolved Nitrogen	TDN [mg/ gDW]
Fungal Necromass-C	FNC (mg/gDW)	Particulate Organic Matter	POM (g OM/kg)
Bacterial Necromass-C	BNC (mg/gDW)	Mineral Associated Organic Matter	MAOM (g OM/kg)
	TMNC (mg/gDW)	Total Organic Matter	Total OM <2mm (g OS/kg soil)
Glucosamine/Muramic acid	Gluc/MurM		

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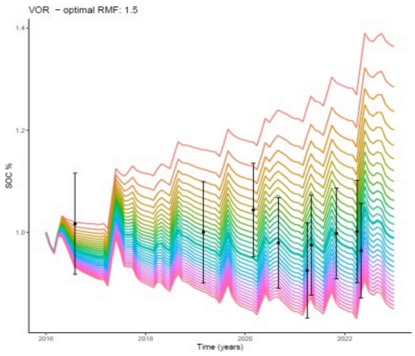
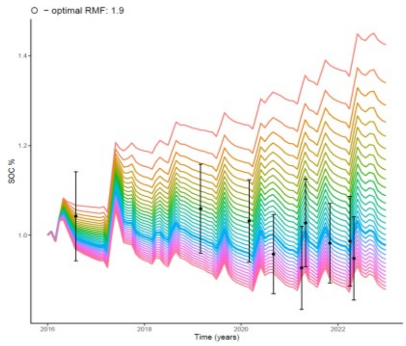
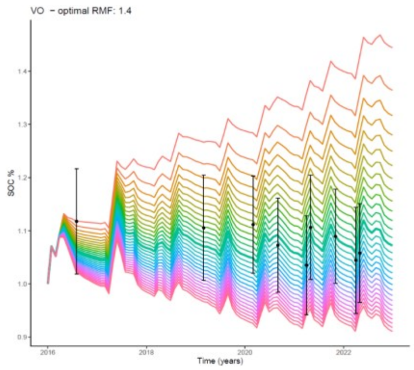
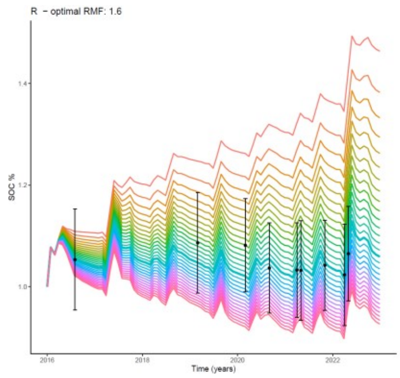
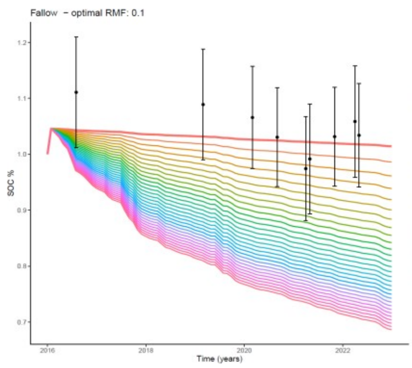
Annex 2. Modelling results treatments in LTE Clever Cover Cropping.

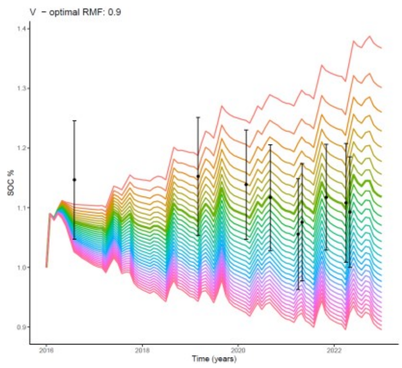


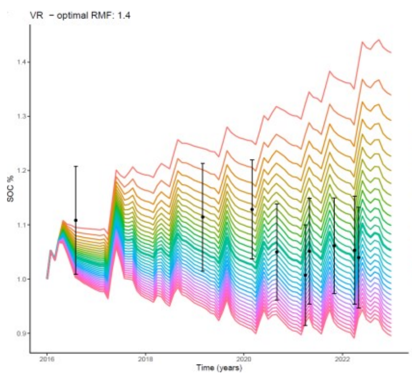


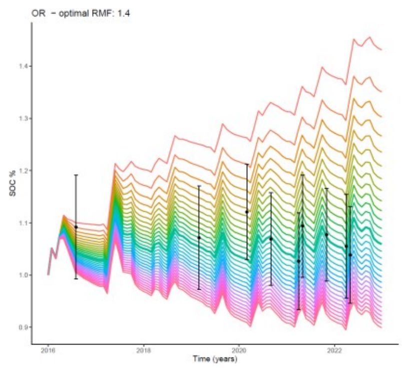












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PART 2.

3. Modelling with the Millennial model

3.1 Adaptation

The original goal of the Millennial model (Abramoff et al. 2018, 2022) was to update early models, such as Century and Roth-C with the goals to (i) define C pools which would be related more directly to field measurements and (ii) reflect current understanding of soil microbial and physicochemical processes. In that regard, the Millennial model includes explicit representation of microbial activity, association with minerals via sorption, and aggregation or organic matter (see Figure 1). A more detailed description of the model, as well as changes and adaptations can be found in Abramoff et al. (2018, 2022).

The interest of using this model in this project was the consideration of different measurable C pools by the model. As some of the pools are considered more stable, i.e., C that is associated with this pool is less likely to be lost due to microbial activity and will thus contribute to C accrual in the soil, our focus was on the question in how far the usage of cover crops might contribute to the buildup of those pools and, with that, help answering the overall question posed in the EnergyLink project, i.e., if higher crop diversity could help increasing SOC.

The Millennial model considers five measurable pools in particular (Abramoff et al. 2018):

- Particulate organic matter (POM), i.e., plant material, dead insects, fungi and detritus
- Low molecular weight C (LMWC), including root exudates, leaf leachate, and the by-products of exoenzyme activity
- Aggregate C (AGG), i.e., C that is attached (or enclosed in) soil aggregates and thus more protected from decomposition
- Mineral-associated organic matter (MAOM), i.e., organic matter that is attached to mineral surfaces via a variety of sorption mechanisms, and
- Microbial biomass C (MIC), i.e., the mass of C contained within soil microbial cells.

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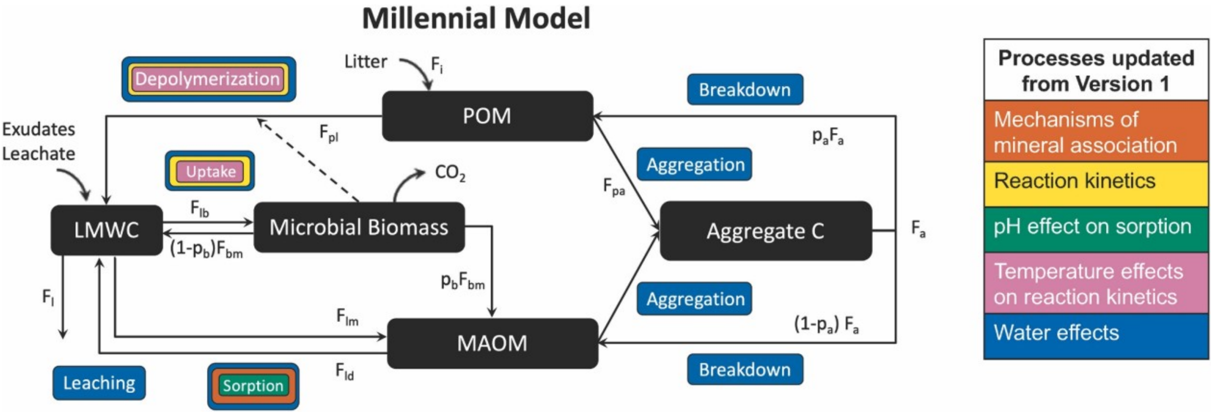

Figure 1. Conceptual model of the Millennial Model (Abramoff et al. 2022). Solid arrows indicate fluxes between pools. Colored boxes indicate modeled processes that have been updated or changed from the earlier version firstly presented by Abramoff et al. (2018). The dash line indicates that microbial biomass controls the depolymerization rate. POM = particulate organic matter, LMWC = low molecular weight carbon, MAOM = mineral-associated organic matter.

3.2 Application

The Millennial model was used to simulate the distribution and development of different C pools at the long-term field experiment Mellby, which is located in Halmstad, southwest Sweden (56°29´N, 13°00´E). The experiment is established on a sandy loam. Mean temperature from 1961 – 1990 was 7.2 °C and the mean annual precipitation was 803 mm. The experiment consists of spring cereals under three fertilization regimes (low rate of slurry, high rate of slurry, mineral fertilizer) combined with presence or absence of cover crops (e.g., Liu et al. 2012, Ulén et al. 2006).

For the purposes of EnergyLink, we considered treatments that received mineral fertilizer with and without cover crops. Of the six plots that fall within this category, two (one with and one without cover crops) were chosen for modelling purposes.

The Millennial model can be run using the R software. Input data needed comprises information on the soil´s pH, bulk density, and clay + slit content. The slope of the relationship between mineral C and clay was taken from the review published by Georgiou et al. (2022) (Table 1).

Table 1. Treatment-specific input parameters used to run the Millennial model.

Parameter	Value
pH [-]	6
Bulk density [kg soil/m-3]	1.22 (control), 1.26 (cover crops)
Slope of mineral C to clay relationship [-]	0.86
Clay + silt content [%]	23
Runtime [yrs]	8 (2922 days)

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Moreover, daily data on soil temperature, soil water, as well as C inputs have to be provided. As the continuous parameters have not been measured in the field, we used the soil-crop model CANDY (Franko et al. 1996; https://www.somod.info/candy_main.php) to estimate variations in temperature, moisture, as well as C inputs (Figure 2).

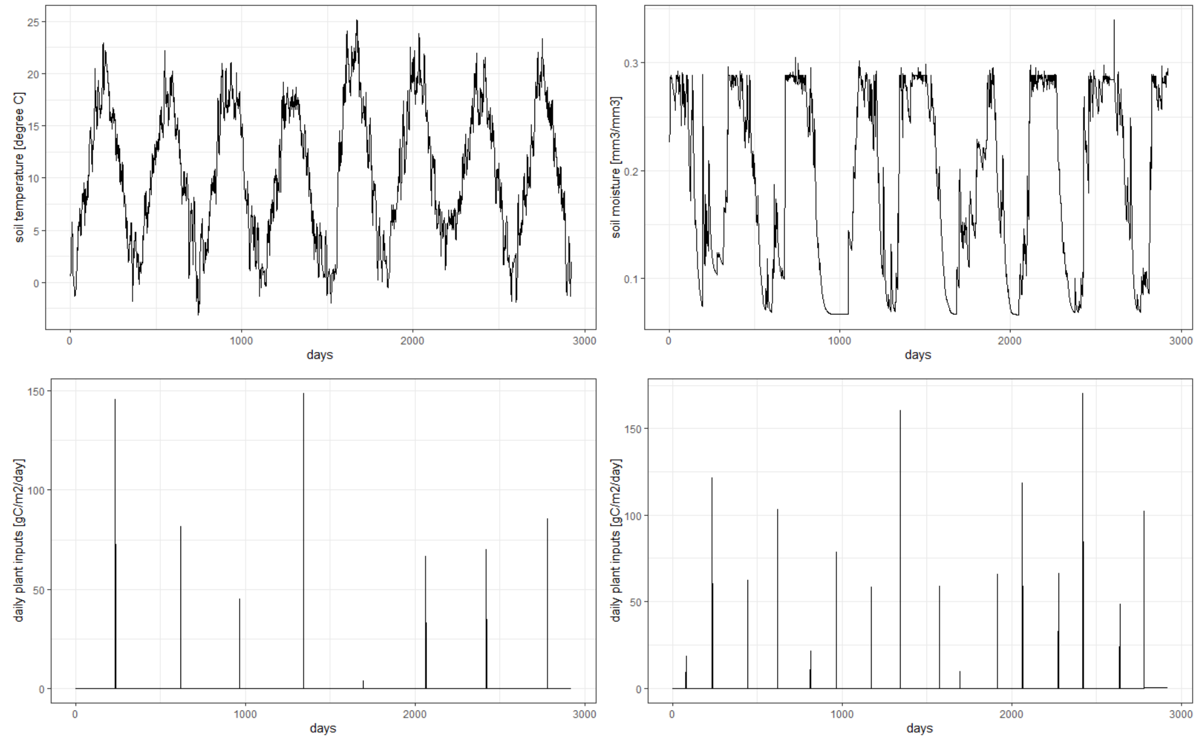

Figure 2. Input data on soil temperature, soil moisture and daily plant inputs for the control (bottom left) and cover crop treatment (bottom right) as estimated with the CANDY model used to run the Millennial model.

As a starting condition, the individual pools were set to a value of 1, which allows to follow the dynamics over time in a comparative manner. One exception are the CO_2 emissions, which have been set to 0. This is due to the fact that the model provides cumulative emissions as an output and it can be expected that these values will not fall below 0.

a. Results

The first simulations were under the assumption that the system was not in steady state, i.e., changes in pools and amount of CO_2 emissions cannot be interpreted as influenced by the treatments but rather as a reaction of the soil system to the establishment of the experiment. These results provide a good insight into the model structure and how the linkages and relationships between pools are simulated. As can be seen in Figure 3, the MIC pool is comparatively unreactive and does not show big changes over time, regardless of the presence or absence of cover crops. Starting from 1, the MIC pool varied between 0.35 and 2.16 g C m^{-2} for the control and 0.57 and 4.06 g C m^{-2} for the cover crop treatment.

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The dynamics, however, followed the C inputs with increases after every input and subsequent decreases. The same applies to the LMWC, even though the increases and decreases are far more abrupt compared to the MIC pool. In contrast to MIC and LMWG, the POM pool slightly increases over time with more inputs and thus a clearer increase in the cover crop treatment compared to the control. The same is true for the AGG and MAOM. The latter in particular reveals the differences between the treatments and at the end of the simulation period (i.e., after 8 years) it is about twice the size in the cover crop treatment compared to the control (774 vs 402 g C m^{-2}).

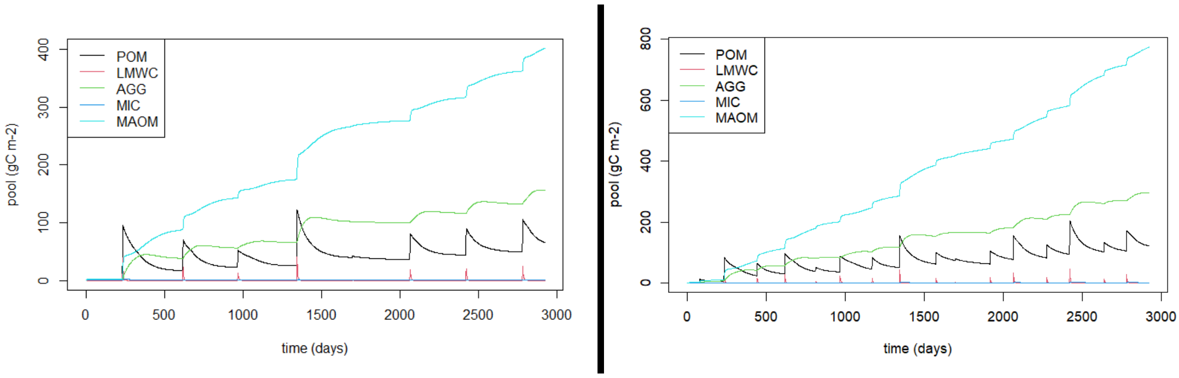

Figure 3. Dynamics of pools following different C input treatments (left = control, right = cover crops) over an 8-years period.

Emissions of CO_2 as well showed a clear pattern in appearance and height as predetermined by the inputs (Figure 4). Again, differences in inputs, i.e., between treatments, resulted in more frequent and higher CO_2 emissions in the cover crop treatment. Total CO_2 emissions accumulated over the 8-year period were 36 g C m^{-2} for the control and 113 g C m^{-2} for the cover crop treatment.

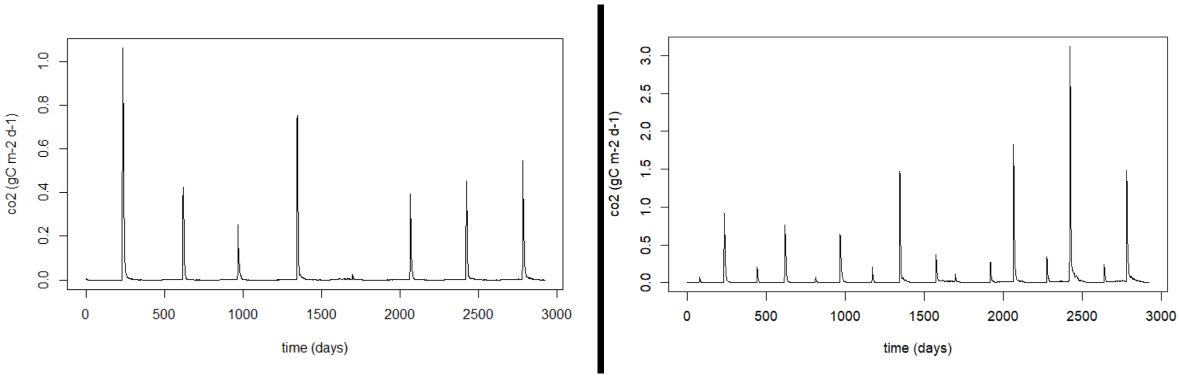

Figure 4. Daily CO2 emissions simulated for the control (left) and cover crop treatment.

In a next step, we assumed that the system has been in steady state, i.e., that there has been an equilibrium between the C inputs and the outputs. To achieve this, the model has been run in a so called spin-up period of 1000 years. This period has not been taken into account for the further analysis of the data.

As can be seen from Figure 5, the individual pools are clearly separated in terms of their size and are rather stable over time, regardless of the treatment. As expected, most of the C is comprised in the MAOM pool, followed by the AGG pool and the POM. The LMWC and MIC pools are smallest in size,

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but in contrast to the larger pools, they still show some kind of dynamics following the C inputs (data not shown).

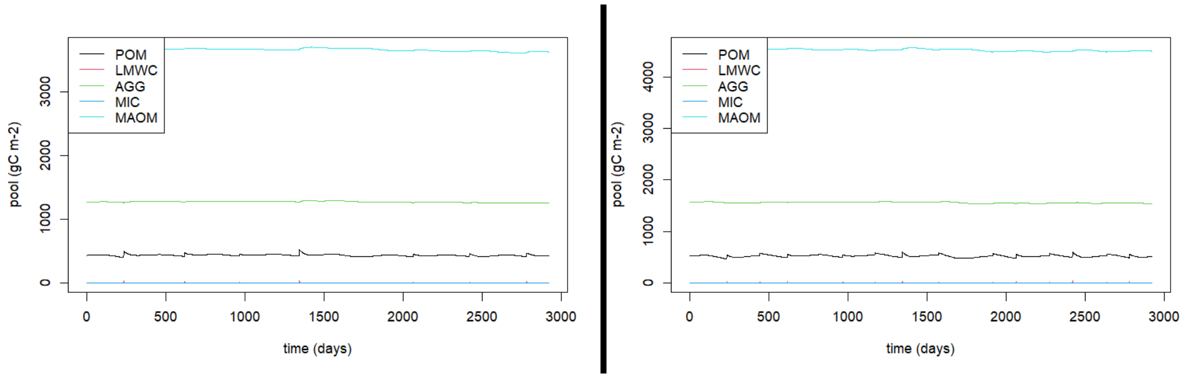

Figure 5. Dynamics of pools following different C inputs (control (left) vs cover crops (right)) over an 8-years period, assuming the system to be at steady-state.

For CO_2 emissions, dynamics and peaks still follow the input patterns, but the heights of the emissions are higher compared to the non-steady state simulations (Figure 6). In fact, total emissions amount to 773 and 1415 g C m^{-2} over the 8-year period.

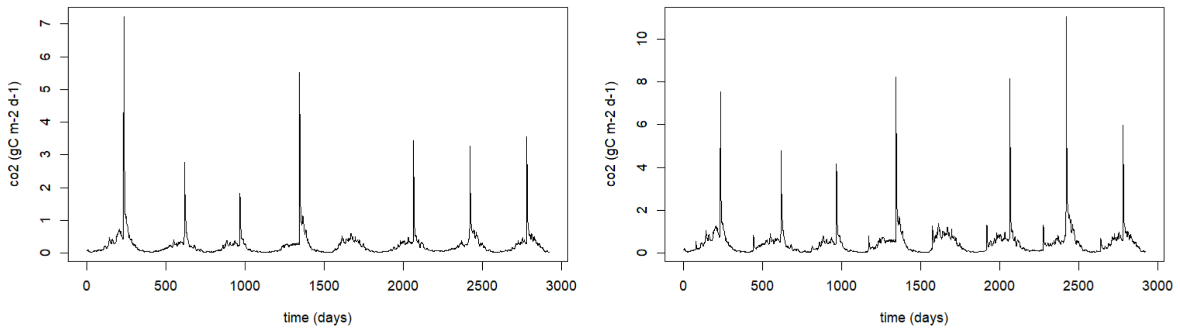

Figure 6. Daily CO_2 emissions simulated for the control (left) and cover crop treatment, assuming the system to be at steady-state.

b. Discussion

This is, to our knowledge, the first study that applies the Millennial model to an actual field experiment comparing different C inputs (presence and absence of cover crops). In this study, we were limited to one field experiment that that considered either one or two crops. Using the model, we simulated the different pools in two ways: (i) without spin-up period and (ii) assuming the system to be in steady-state. The actual field experiment in Mellby has been running since the 1990´s and it cannot be expected that the system is in steady-state already. However, following the model, the individual pools can be expected to react different already and the higher plant and C input provided by the cover crop treatment lead to a buildup of the MAOM and AGG pools in particular (Figure 5). The pools are the ones known to comprise higher contents of SOC compared to the POM, MIC and LMWC pools. This is primarily due to the fact that organic material is bound either on top or within the structure of soil aggregates (AGG) or by a variety of sorption mechanisms, such as surface complexation, cation bridging and hydrophobic interactions (Sollins et al. 1996, Kaiser et al. 1996, Kleber et al. 2007, Torn

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et al. 2009). According to several studies, MAOM accounts for up to 85 % of the total SOC stock in bulk soil and has been found to have a longer mean turnover time than e.g., AGG and POM (see Abramoff et al. (2018) and references therein). The conceptual organization of the Millennial model, i.e., the clear definition of individual pools that can be measured in the lab, replaces older concepts that assume mainly three pools with rather fixed turnover times. In the first presentation of the model, t al. (2018) compare Millennial with the Century model, which considers an "active" pool (turnover time 6 months - 1 year), a "slow" pool (10 – 50 years) and a "passive" pool (100 – 1000 years). However, the approach used in Millennial is more dynamic and based on measurable pools. In that way, the contribution of microorganisms and microbial activity is implemented. Microbes transfer mass between all of the C pools, meaning that the model provides the potential to generate a wider range of feedback in response to climatic changes compared to a simpler first-order model. In fact, even though the microbial biomass per se is not a large pool in the soil (< 5 % of SOC according to Fahey et al. 2005, Fontaine et al. 2007 and Abramoff & Finzi 2016), microbial activity strongly reacts to climate change and thus has a strong effect on C cycling. The MIC pool is this study was smallest compared to the other pools, but its dynamic and size followed the treatments and, more specifically, inputs of C. Even though the C losses, as expressed by CO2 emissions, were higher under the cover crop treatment, the overall higher amount of inputs led to larger C pools and, as a result, SOC accrual as a whole.

Nevertheless, the input data to run the model is rather complex and while there often is data for soil moisture and temperature, information on (in particular daily) C inputs are often not measured. In this study, we used another, well-established, carbon model to estimate the inputs of C to be used to run the Millennial model. In addition to this, even though novel technology allows for fractionation of organic carbon, these analyses are often costly and until now only seldom available. The information available for the Mellby experiment does not deem sufficient for an in-depth analysis of the model, as data for validation is missing. Still, from a process-understanding point of view, including microbial processes, i.e., transfer of C between pools, will be of high importance for future predictions and considerations of climate change, as the response of microorganisms to elevated soil temperature is well-known and can be expected to alleviate C turnover. We did not perform a proper calibration of the model to the site, mainly due to the lack of available data. From analyses run in the *EnergyLink* project, we know that there are no significant differences in either SOC or carbon use efficiency (CUE) between the treatments established at the Mellby site (Schroeder et al., under review). This means that even though the consideration of microorganisms and microbial activity in biogeochemical models seems the right way to go, for Mellby, it might mean that the effect of cover crops on SOC accrual will be overestimated.

c. Conclusion

This is the first application of the Millennial model to a Swedish field experiment on the effects of cover crops. In that, the model clearly shows the benefits of additional carbon input provided by cover crops, on the development and size of individual (measurable) C pools. In practice, these benefits have not been observed (yet), which leaves the question for the need of a more explicit description of microbial activity and carbon use efficiency open for discussion.

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