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Modelling the Organic Evolution of a Mediterranean Limestone Soil under Usual Cropping of Durum Wheat and Faba Bean

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Abstract: The modeling of carbon (C) and nitrogen (N) fluxes between microorganisms and plants in pure and associated cultures of durum wheat and faba bean demonstrated a close link between the C and N cycles in agroecosystems. The MOMOS (microorganisms and organic matter of soils) model integrates simplified descriptions of photosynthesis (origin of organic C in soil), N microbial exchange (soil origin for N), N fixation (atmospheric origin for N), and plant growth with an organic matter decomposition core that has the soil microbial community at its center. This work provides estimates of the exchange parameters between plant organs and microbes, which were compared to literature data when available. In a connection with photosynthesized C, the root demand for inorganic N can be adjusted by its microbial production. Our approach is a new methodology for improving plant production, by optimizing the interactions with soil microorganisms. Additionally, the coupling of plant growth and microbial processes enabled determining changes of the organic compartments of soil. In the unfertilized limestone soil of this study, sequestration was found to be located in the labile microbial metabolites for one year, then significantly transferred to stable humus during 6-year intercropping. Thus, we propose the MOMOS mathematical tool, not only for guiding ecological intensification, but also related to the management of agroecosystems for climate change mitigation.

Keywords: carbon cycle; nitrogen cycle; modeling; MOMOS model; C sequestration; climate change

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1. Introduction

Models that link more accurately the carbon (C) and nitrogen (N) cycles have long been sought after to predict the transfers between the organic and inorganic compartments of plants and soils [1–3]. Many recent research works have focused on modeling the long term evolution of OC stocks connected with different variables and practices: the effects of crop rotation [4,5], afforestation [6], soil temperature [7], farming systems [8,9], bioenergy crops [10], fire and drought [11], tillage and N fertilization [12], organic amendments, and forest systems [13]. The considered evolution periods varied between 4 years [10], 5 years [6], 10 years [4], 12 years [11], 13 years [12], 18 years [13], 27–28 years [14], 30 years [5], and 50 years [8,9]. Some other models have focused on plant production, nitrogen (N) cycle, and N fixation over shorter periods [15–17]. However, none of the models used were really based on the functional ecology of microorganisms in the short term and in relation to climate conditions and plant production.

In contrast, the MOMOS model (modeling organic transformations by microorganisms of soils) is centered on the functions of feeding, respiration, and mortality of microorganisms. Initially proposed for a comparative prediction of ^{14}C flows in tropical soils [18], it

has since been mathematically related to climate data, the biological properties of plant residues, and soil data [19,20]; then, validated [21] and extended to the prediction of ^{15}N flows in six contrasted tropical ecosystems [22]. The challenge was then to link microbial dynamics to plant growth and to determine the transfers between soil, atmosphere, plant organs, and the microorganisms regulating the continuous C [23,24] and N flows [25]. In global change studies, may MOMOS be considered a predictive tool for the balance between the residual inputs and respiration losses in soil, as well as the humification dynamics resulting from microbial mortality? (HL and HS in Figure 1). The challenge was to predict data obtained from short-term assays to evaluate quantitatively continuous microbial transformations, when other models require a much longer time (see above). We chose field trials with unfertilized durum wheat/faba bean intercropping on a Mediterranean calcic soil, which would allow focusing on the natural transfer mechanisms in the absence of fertilization or chemical treatments. We used a hybrid approach, where crop-specific model parameters were calibrated, while the parameters that determine soil organic matter decomposition were preserved from previous studies. Was it possible to calibrate the transfer parameters between plant organs and microorganisms from the measurement of the state variables (C and N stocks of living organisms and soil)? The additional challenge was to assess the applicability and robustness of MOMOS under Mediterranean conditions, which are very different from the tropical conditions used to propose and validate this model. A further aim was to test the model sensitivity for global change evaluations, by simulating 6-year replicates of the cropping season used for calibration, giving a view of the organic evolution of the soils and crops generally used in the Mediterranean region.

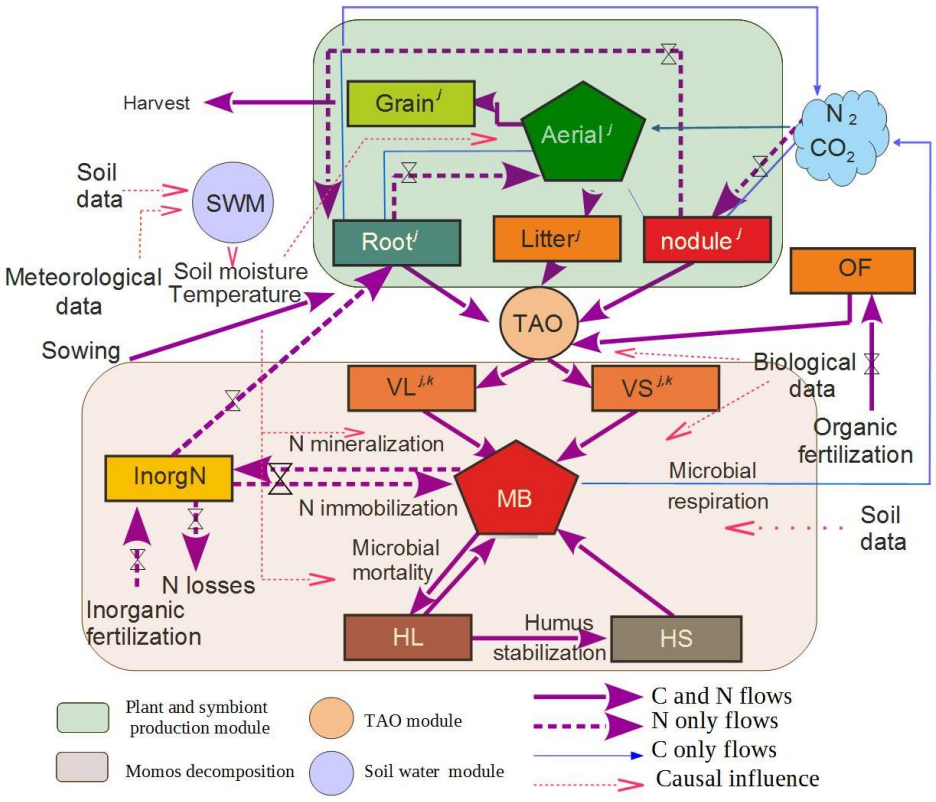

Figure 1. The MOMOS model (microorganisms and organic matters of soils): coupling the decomposition and vegetal production in connection with soil climate, biological data (TAO, transformation of added organic matters), and soil texture. MB is microbial biomass, VL^{j,k} and VS^{j,k} are labile and stable fractions of each organ k of plant j or organic fertilizer OF. HL and HS are labile and stable humus, respectively, inorgN is the inorganic N continuously exchanged with the MB and roots of plants. SWM is the soil water module associated with organic transformation.

2. Materials and Methods

2.1. Experimental Area

The field experiments were carried out at a station of the National Research Institute for Agriculture, Food, and Environment (INRAE) located at Mauguio (south-east of Montpellier, France). The climate is Mediterranean and includes hot and dry periods. The soil is a calcic chromic Calcisol (WRB, 2015), which is typical in many Mediterranean areas. It is developed on Pliocene calcareous molasses. The brown-reddish upper layer (~60 cm thick) of the soil was partly decarbonated ($CaCO_3$ < 2%, pH~8.2). The texture was loamy (USDA triangle) with 21.9 ± 3.6% (mean ± SD) 0–2 µm clay, 25.2 ± 2.4% 2–20 µm fine silt, and 21.5 ± 1.1% 20–50 µm coarse silt. The cation exchange capacity (CEC) was 21.8 ± 1.2 cmol kg^{-1}. The carbonate content was 1.7 ± 1.2% of $CaCO_3$ and the pH was alkaline (8.2 ± 0.1)

The plots had not received any fertilizers for 13 years. Additionally, they were not irrigated or treated with any herbicides or pesticides. Weeds were removed by hand, with evaluation of their C and N stocks, and the yields were poor.

2.2. Field Experiment

Twelve plots of 6 m × 10 m were used, with four field replicates of three modalities: (1) a durum wheat cultivar alone, (2) a faba bean monocrop, and (3) a faba bean/durum wheat intercrop. Four whole plants (roots and shoots) of durum wheat and four of faba bean were collected from each plot during growth (1st sampling period). Then, 10 plants of each species were taken during flowering (2nd sampling period), and four of each species were taken at maturity (3rd sampling period). The roots, aerial parts, and grains of plants were weighed separately after drying and their C and N content was measured. The symbiotic nodules of faba bean were also separated, carefully washed with deionized water, weighed, and measured for C and N. Samples of the near-root soil were collected and stored briefly at 4 °C for determination of the microbial biomass (MB-C and MB-N) by fumigation-extraction [26] and measurements of total C and N. Meanwhile, two replicates of soil samples from the 0–5 cm and 25–30 cm layers were collected in 500 mL stainless steel cylinders from each plot. These samples were used to determine the soil moisture and bulk density in order to convert the soil C and N concentrations into C and N stocks (g m^{-2}) in the 0–30 cm soil layer, assuming it was the layer with the C and N exchange of the roots and microorganisms. Plant densities were measured in the field and used to convert C and N concentrations into the C and N stocks of each plant organ or symbiotic nodule by m^2. A LI-COR flux chamber measured soil CO_2-C respiration converted into g C m^{-2} day^{-1} three times in the cropping season on each plot (4 replicates per plot).

2.3. Modeling the C and N Exchanges

MOMOS is based on the functional ecology of the soil microbial biomass (MB), which is increased by the enzymatic assimilation of labile and stable vegetal necromass (VL and VS) and labile and stable humus (HL and HS) and decreases with microbial respiration to atmospheric CO_2 and mortality to labile humus (Figure 1). The only process that is assumed to be more chemical than biological is the low humus stabilization from HL to HS. The MOMOS microbial core (pink rectangle in Figure 1) is only parameterized by the seven kinetic parameters included in the matrix $\mathbf{A_e}$ of the MOMOS core general equation, describing the behavior of each element $\mathbf{e} \in$ [C, N] exchanged with the microorganisms:

$$\dot{\mathbf{X}}_\mathbf{e} = f(T)f(\theta)\,\mathbf{A_e}\,\mathbf{X_e} + \sum_j \sum_k \mathbf{B}_\mathbf{e}^{j,k} \quad (1)$$

where $\mathbf{X_e}$ is the vector of the state variables (C or N content of each of the five organic compartments VL, VS, MB, HL, HS of Figure 1), $\dot{\mathbf{X}}_\mathbf{e}$ is the vector of output flows from each organic compartment (the derivatives of xe), $\mathbf{A_e}$ is the model parameter matrix, which uses the same parameters for each element $\mathbf{e} \in$ [C, N], except the central element of $\mathbf{A_e}$,

which expresses microbial respiration for C using the proposed quadratic law [18], which expresses N microbial exchanges by mineralization or immobilization [25].

$\mathbf{B_e}^{j,k}$ is the vector determining the external C and N inputs from death of an organ or symbiont *k* of any plant *j* and possibly from organic fertilization. *f(T)* and *f(θ)* are the correcting functions of temperature and soil moisture [21].

Complementary equations were used to link the microbial respiration to soil texture or possibly to soil pH [20], as well as to link the microbial assimilation and mortality to the C-to-N ratio of debris substrates [19]. Most of the C and N flows were predicted by the same decomposition parameters (violet thick solid lines in Figure 1) in the equation system showing the C:N ratio of each dead plant organ $\mathbf{B_e}^{j,k}$ entering decomposition (which can be stored in a database of plants). Two N specific flows (violet thick dashed lines in the pink rectangle of Figure 1) express exchanges of inorganic N with MB decomposers by microbial immobilization and microbial mineralization. Two other N specific flows (violet thick dashed lines in Figure 1) concern (i) the adsorption of inorganic N by the roots of plant *j*, and (ii) N fixation by root microbial symbionts, then its transfer into plant organs. Three flows are C specific (thin solid blue lines in Figure 1). Two of them express microbial respiration adjusted by the MOMOS quadratic law and the root respiration associated with each plant species. The last specific C flow gives the atmospheric CO_2-C transfer to plant leaves by photosynthesis. One aim was to attempt to preserve the equations and parameter values directly related to microbial decomposition established from ^{14}C and ^{15}N tracer experiments in tropical conditions [18–22], (pink rectangle in Figure 1) by introducing only the new soil and climate conditions.

The other objective was to attempt to calibrate the transfer parameters of the plant and symbiont production nodule (large light green rectangle of Figure 1) that regulate the flows of C from the atmosphere to the plant leaves (photosynthesis), the flows between inorganic N and microbial decomposers (immobilization and mineralization), the flow of N from the soil to the plant roots (root adsorption), the flow of N between the atmosphere and the plant symbioses (N fixation), the flows of C and N from the grain sowing and senescence (germination, natural mortalities, weed removing, and harvest), and the transfer parameters between the plant organs. The goal was to calculate these transfer parameters by fitting the model to the measurement of C and N stocks in the living organisms (green rectangles, red rectangle, and red pentagons of Figure 1), the total soil C (sum of polygons of the pink rectangle of Figure 1), and the C respiration (thin blue lines of Figure 1) from the field experiment.

3. Results

3.1. Robustness of the Microbial Core of MOMOS

One main result, which was demonstrated previously [23–25], was that the parameters of the pink rectangle in Figure 1 defined and calibrated in tropical rainy conditions and acidic soil could be preserved in this study in semi-arid Mediterranean conditions on calcareous soils. This is a proof of the robustness and transferability of the MOMOS core method. The relationships linking these parameters to meteorological data, soil texture, and quality of residue inputs were also preserved. Only the new Mediterranean data on meteorology, soil texture, and residue quality had to be entered to obtain accurate predictions. This proves the model's capacity for being extended over large areas of the Earth, only requiring the new data on soil and climate.

3.2. Transfer Parameters Regulating Plant Growth

The other main result that was demonstrated previously [23–25] is that the parameters that connect the microbial core to plant organs and regulate the transfer between these organs and the atmosphere and soil (arrows of green rectangle in Figure 1) can be adjusted to predict the measured state variables. This was done by fitting new transfer rate parameters (τ in [24] and [25]) and time functions (*f* [24]) of principal importance to understand and optimize the plant growth and crop yields of the agro-ecological system. These predictions,

which are partly reproduced in Figure 2a,b, accorded with literature data, when available. However, sometimes there was no data, because the parameters are difficult to estimate by the other measurement methods, such as tracer experiments in the field. Therefore, we proposed our modeling approach, converting the state variables to flows for generalization in field conditions. This study gave a unique set of parameter values, whatever their initial conditions, indicating that the model is not over-parameterized. Applying this proposition to various systems will be key for a new intensification of yields, plant growth, and crop production, while respecting the environment.

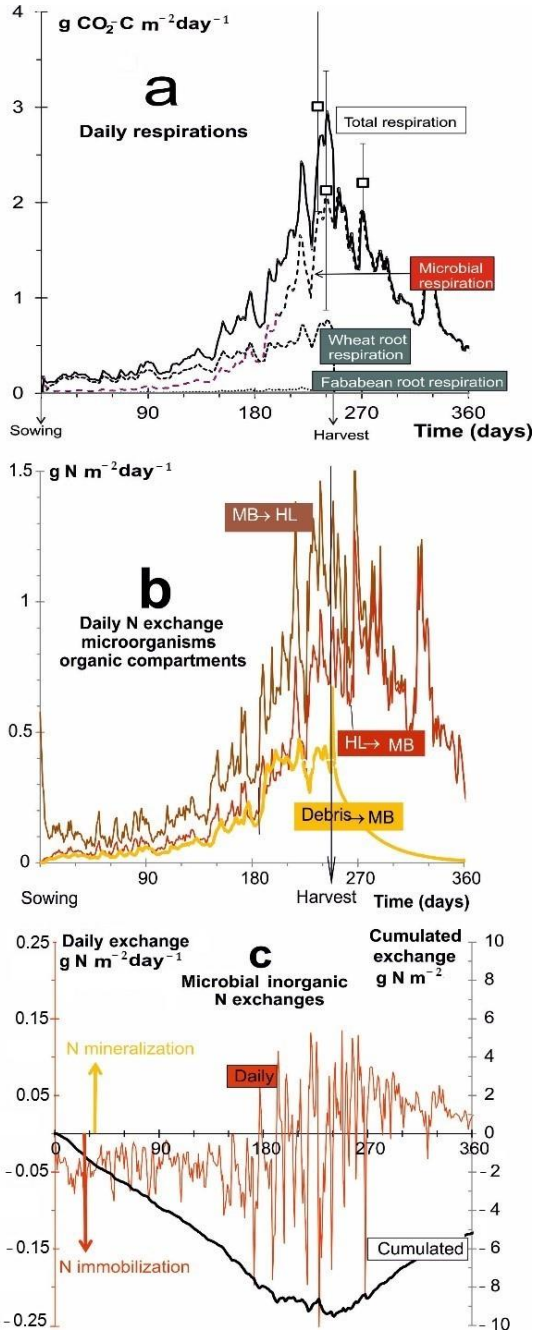

Figure 2. Example of flow predictions during intercropping: (**a**) bold black line: total daily respiration compared to field measurements (open square), red dashed line: microbial respiration, olive green dashed line: root respirations, (**b**) continuous exchanges of organic N with MB, (**c**) continuous exchange of inorganic N with MB.

3.3. Continuous Predictions of Organic Evolution of Soil

Predictions of C and N stocks of the MOMOS state variables (Polygons of Figure 1) correlated significantly with the measured values and fell inside the 95% confidence intervals of these values (Example of Figure 2a). The flows estimated by the model were consistent with literature data, when available. These are essential for our understanding of C and N exchange and for finding ways to improve the performance of agricultural systems, for example to minimize the C and N losses in the root system in the case of cereal cropping.

The extension of the results obtained in a cropping season to longer evolutions of C contents of soil submitted to the same cropping enabled prediction of the behavior of the labile and stable C compartments (Figure 3). MOMOS is thus a key tool to predict, not only yields, but also soil C contents, sustainability, and the ability of systems for carbon sequestration and mitigation of climate change.

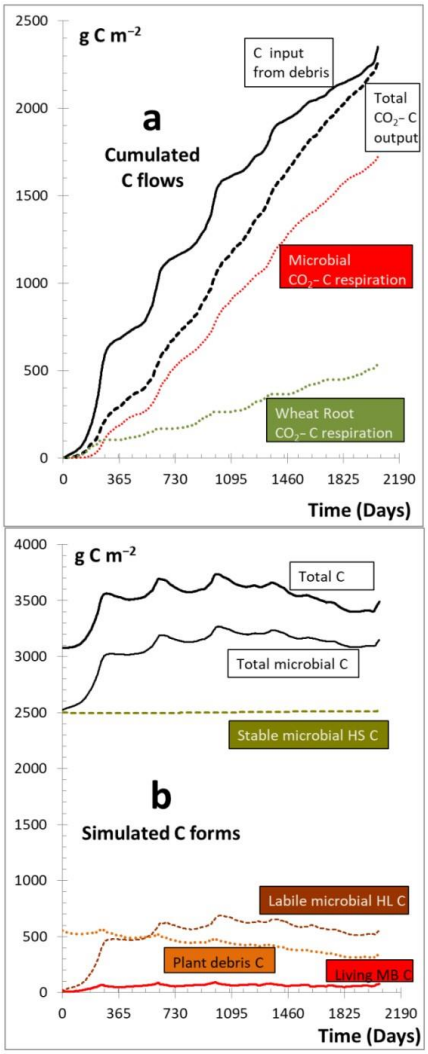

Figure 3. Prediction of the C evolution of the experimental Mediterranean soil after 6 years of unfertilized cereal/legume intercropping: (**a**) accumulated C inputs from photosynthesized debris (black continuous line) compared to accumulated outputs by respiration (black dashed line) of microorganisms (red) and plants (olive green), (**b**) simulated C during 6-year intercropping, total C and microbial C (plain black lines) originating from plant debris (orange dashed line), living microorganisms (red), labile humus (HL, Brown), and stable humus (HS, green grey).

4. Discussion

4.1. Flow Simulations during Intercropping

The model was able to decompose the total flow of respiration into microbial respiration regulated by the MOMOS microbial core (pink rectangle in Figure 1) and plausible values of root respiration for each plant, depending on the balance between photosynthesis and the C content of plant organs (Figure 2a). In our intercropping implementation the cereal respiration was found to be relatively high, and this was probably due to the low fertility of the system. The CO_2-C root respiration should express the energy lost by the cereal in order to find N (and other fertilizing elements) in the soil. This is probably the mechanism explaining the low yields of cereals when the soil fertility is poor. Conversely, the legume root respiration was adjusted to near zero, indicating that these plants do not need to explore the soil with their root systems. N is delivered by atmospheric fixation by symbiotic bacterial nodules.

Other modeling outputs (Figure 2b) illustrated the high flows of organic N crossing the MB. Analogously to the cellular functioning of the human and animal body, the MB continuously absorbs all organic N from the available substrates: VL vs. plant debris and HL and HS humus. However, the greater part of this N is continuously rejected into the environment, by microbial mortality in the case of MB, and probably also by cellular death in the case of the human body. Our study showed that less than seven per mile of organic N inputs were stored in MB (see Figure 3c of [25]) and were then possibly partly rejected as inorganic N.

In contrast with organic exchanges of N (Figure 2b), the flows between inorganic N and MB (Figure 2c) were found to be about ten times lower. The microbial immobilization depended on the threshold of C-to-N ratio of MB. A MB C-to-N ratio higher than the threshold value indicates a N demand by the microorganisms that induces net microbial immobilization. Conversely, a low microbial C-to-N ratio induces net N mineralization. This shows that the mechanism of crop yield is increased by organic N fertilization. The low C-to-N of the fertilizer induces a low C-to-N value in the MB and consequently, the mineralization of inorganic N available for plant roots. The high oscillations in Figure 2c illustrate that the available inorganic N is greatly dependent on the meteorological conditions.

Net microbial mineralization occurred when the values of inorganic N were positive (Figure 2c) and available for the plant roots. Conversely, negative values showed a microbial immobilization, which continuously lowered the N transfer to the plant roots and crop productivity. This study in an unfertile system showed a predominance of microbial immobilization during, at least, the first six months of plant growth. The cumulative immobilization peaked around 9 g N per m^2 before harvest and then decreased due to mineralization, mainly of legume residues up to 5 g N per m^2. The symbiotic fixation of atmospheric N was found to approximately compensate the losses of N available to the plant roots by microbial immobilization. The fixation of atmospheric N improved the legume growth and was transmitted to the soil by legume mortality; in particularl, the mortality rate of faba bean roots was optimized at a high value compared to that of durum wheat. The N transmitted from the dead faba bean to the MB engendered a positive N microbial mineralization in the second phase of intercropping, giving inorganic N that was possibly usable for cereals. However, in this experiment, this inorganic N was delivered too late for a possible benefit to the cereal.

4.2. C Sequestration in Mediterranean Limestone Soil

An interesting aspect of the modeling approach was enabling simulations of a given situation for a given time. The MOMOS simulations of 6-year replicates of the experimental cereal/legume intercropping predicted the C evolutions of Figure 3. Meteorological data were measured on the site, while other data are replicates of the 2011 cropping season.

The cumulated C flows for six cropping seasons were predicted up to 2300 g C m^{-2} (Figure 3a), near the total-C of Figure 2b. The flow of the C inputs from plant debris was found to be always greater than the accumulated CO_2-C losses by microbial respiration,

but the difference tended to decrease at the end of the simulations. About 80% of the CO_2-C outputs originated from microbial respiration, while 20% originated from cereal respiration. Perhaps this latter result could be lowered by improving the intercropping system (see Section 3.1) in such a way that the cereal can benefit from the atmospheric N fixed by the legume symbionts (see Section 4). Thus, the cereal roots would not have to lose so much energy to find inorganic N in the soil.

The sequestration of total C in the soil peaked at about 600 g C m^{-2} in the 3rd cropping season then decreased to almost 300 g C m^{-2} at the end of the 6th season (Figure 3b). These values, corresponding to 6 and 3 tons C ha^{-1}, indicate the significant contribution of this unfertilized intercropped system to global climate change mitigation. This result needs to be confirmed with other cropping systems, in order to evaluate, and possibly improve, the process for all Mediterranean areas.

Most of the total C in the soil originated from C synthesized from microorganisms (total microbial C in Figure 3b). The total C minus microbial C represented the stable vs. the fraction of vegetal debris not yet assimilated by microorganisms (orange dashed line in Figure 3b). This fraction decreased slowly, by about 40% (550 to 350 g C m^{-2}), during the 6-year simulation. It would be interesting to compare the predictions of C values of the coarse fractions separated by the physical fractionation [19].

In the short term, the greatest part of the C sequestration was in the labile humus HL, mainly produced by microbial mortality. The HL predictions increased from almost 500 g C m^{-2} during the 1st cropping season to about 750 g C m^{-2} after the 3rd season, then decreased slowly to about 500 g C m^{-2} after the 6th season. HL is the main reservoir of fertility for further plant growth, as shown in the intense N turnover between MB and HL (Figure 2b).

During the first year of intercropping, the labile humus HL was the only reservoir of C sequestration, despite it representing only 20% of the C of microbial origin. Indeed the greatest reservoir is stable humus HS, which was found to be approximately constant during the 1st cropping season; with a slight decrease from 2499 to 2494 g C m^{-2}. However, the 6-year predictions showed a slight increase of HS, up to 2513 g C m^{-2}. This sequestration of 14 g m^{-2} of the stable C could represent 2300 g C m^{-2} after 1000 years; close to the 2500 g HS-C m^{-2} of the modeled HS. It would be interesting to compare long term MOMOS predictions (C amount and C age) and ^{14}C dating of stable C [27], after removing the labile C fractions [19].

Future research to ecologically improve the yields of cereal/legume systems must focus on early flowering legumes in association with late flowering cereals, or sowing the cereal later than the intercropped legume, or rotations of legume/cereals in place of intercropping.

5. Conclusions

The proposed equation system linking the microbial transformations to plant growth accords with predictions of the state variables measured in the field. It enables the optimization of the parameter values regulating the transfer of C and N through plant organs and symbionts, with a unique set of results, irrespective of their initial values. The modeling methodology enables the estimation of the growth parameters that are essential to agronomy and ecology, but which are sometimes difficult to measure using other field methods. The MOMOS core defining microbial decomposition was also validated by this study and in another experiment in Algeria [28]. All the microbial parameters and their connections to the environmental variables defined by ^{14}C and ^{15}N tracer experiments in acidic tropical conditions can be preserved in calcareous Mediterranean conditions, such that only the new environmental variables must be entered. Agricultural improvements for the studied durum wheat/faba bean cropping were proposed from these results. Extending the simulations from one to several cropping seasons enabled predicting the C and N long term evolution of humified compartments. Therefore, the tool is also proposed for improving global climate change predictions.

Author Contributions: All authors contributed extensively to this work. H.I., N.B., M.P. and D.B. organized the setup of the experiments and collected the data. H.I., S.G., M.P., K.V.d.M., N.B. and D.B. analyzed the data, evaluated the results and drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

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