Simulating Microbial Functional Diversity Dynamics in Agricultural Soils: An Individual Based Modeling Approach

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Abstract

New agricultural soil model approaches based on the microbiome dynamics must be considered as they can contribute to understand microbiological soil processes directly linked to substrate metabolism and the influence of these processes on plant growth. The present work presents an approach to the modelling of the interactions of the soil microbial functional diversity with the plant in terms of functions associated to specific processes of organic Carbon and Nitrogen metabolism. The substrates transformations arising in the organic matter that enters as a part of an agricultural scheme are the base for define this metabolism. As result, it has been possible to simulate a rhizospheric soil based on the concept of complex system dynamics and Individual Based Modeling known too as Agent-Based Modeling in an agricultural management context. The explicit definition of the microbiome functional diversity and the processing of the structural elements Carbon and Nitrogen, allowed representing the functional dynamics of this complex system composed by microorganisms, Carbon, Nitrogen and the plant. The variables that reflect the biology and the adaptation to the rhizospheric environment characterized the microorganisms and the assemblage community patterns in time. The main simulations output are system glucose and nitrate levels and an approach to plant growth, all resultant from the metabolic process of the considered Carbon and Nitrogen consortia. The results indicate that the microorganism's diversity assemblages and its functional expression have a fundamental role in terms of plant growth.

Keywords

System Dynamics, Soil Microbiota, Agricultural Productivity, Microbial Ecology, Netlogo

1. Introduction

Soil microbiota has recently gained increased attention in the context of soils theory, implying changes in old theoretical paradigms regarding soil science and soil biology [1] [2] [3]. The importance of approximations to the modeling of soil metabolism based on a biodiversity point of view is supported by experimental evidence that concludes that a difference in the importance of biodiversity is associated with the level of complexity in the substrate's transformations and metabolisms [3]. This has suggested associations between microbial taxa with specific functional soil responses [4] [5] [6].

The modelling of the microbiota has evolved from black box models or nonstructured models like Monod kinetics expression in which intracellular processes are not explicitly considered, to the structured models in which intracellular and individual ecological process are explicitly represented. One of the techniques used for these new approaches is Individual (IBM) or Agent Based Models (ABM) [7] [8].

Individual based models (IBM) show important advantages in face of other modelling techniques for addressing question related to microbial diversity and the functional implications in the dynamics of the system, with a base in the biological process phenomena [9] [10] [11]. IBM allows the understanding of the interactions between individual organisms and the environment in conjunction with the adaptive behavior at the individual level. Accordingly, the dynamics of the system get articulated to the behavior patterns of each one of the individuals or biological entities in the system [12] [13] generating emergent patterns associated to the ecological structure of the microbiota assemblage and soil important functions [14] [15].

In the field of microbial ecology, IBM has important connotations because this technique has the potential of encompass aspects of the relationship between structure and function in microbial communities [16]. In terms of soil ecological process, one of the biggest impacts of IBM has been in the possibility of representing computationally different edaphic microbiomes that include bulk and rhizospheric soils and the concept of transformations of important soil molecules [17]. Resat *et al.* (2012) proposed a detailed Carbon metabolism model, that should be considered an individual based approximation given the search for the expression of microorganism's populations heterogeneity by means of mathematics, but the model requires the numerical parameterization of an elevated number of equations [18].

An important approximation and a fundamental contribution to the recognition of biodiversity as a structuring element in the soil process was defined by Moore *et al.* (2014), getting the definition of microbiological aspects in soil microbial communities and its association with Carbon metabolism through exoenzymes, establishing a control point that improves the modeling approximations notoriously [19].

Considering Carbon and Nitrogen transformation from a metabolic perspec-

tive, labile Carbon and mineral Nitrogen must be considered as the product of processes that requires the presence of families of exoenzymes associated to specific microbial groups. Microbial groups that process the cellulose and the organic Nitrogen transformation to mineral Nitrogen as it is considered an essential nutrient for vegetal growth and its cycle depends on the synergistic microbiota activity [15] [20]. We propose a novel approach to the modeling of an agricultural soil from an explicit microbial biodiversity perspective representation. The work objective is the representation of the relationship between an ecological context defined by the agricultural system, and the dynamics of microbial populations in the generation of mineral Nitrogen for plant growth in a space of rhizospheric soil by means of IBM.

2. Methods

2.1. System Definition

The purpose of the agricultural system development was the identification of the some of the most relevant causal relationships in the system plant-soil-microbiota within the frame of agricultural production for a subsequent simulation. The agricultural system was considered based on previous agricultural soil models [21]. The fundamental elements that would permit the definition of a "generic agricultural system" were extracted. With these elements and their interactions, the microbiota element was incorporated and disaggregated from a "black box" (Figure 1). Given that the concept of agricultural management deals with a great quantity of interactions between the farmer and the culture,
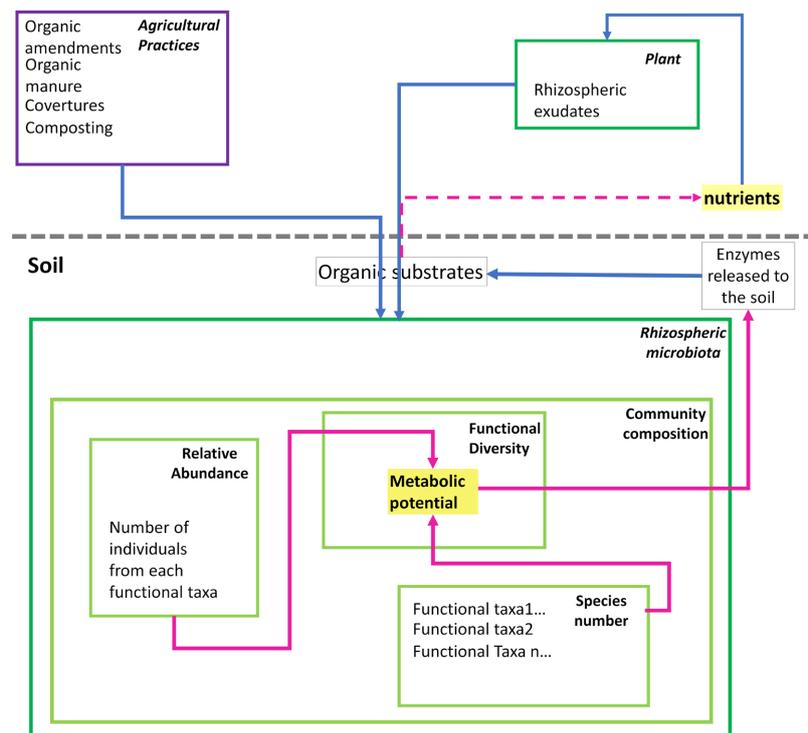

Figure 1. Diagram of the system considered for further Netlogo simulations.

the management is defined based on the input of organic matter to soil, because it allows the development of the rhizospheric soil microbiota and its expression in a simulation. Microbiota is developed inside the system around two fundamental processes, cellulose transformation into glucose and organic Nitrogen processing into mineral Nitrogen bioavailable for plants (Figure 1). Carbon metabolism or the cellulose transformation is based in the work of Choi *et al.* (2018). Nitrogen metabolism is based on a general conceptual frame in terms of mineralization and Kegg. These processes are defined form one aspect of agricultural management, the input of organic matter that could be defined as complex organic Carbon molecules in the form of cellulose and organic Nitrogen in the form of proteins [22].

2.2. System Simulation and Implementation

Individual based modelling (IBM) applies the concept of characterize the simulation different individuals, characterized by variables, in this case, microorganisms characterized by attributes. Specifically, rhizospheric soil microorganisms defined or characterized by biological variables accordingly to the biology of soil microorganisms. The computational simulation of the IBM was developed and implemented in the NetLogo Platform (Wilensky, 1999); the performed experiments in the *Behavior Space* tool of NetLogo considered interactions between variables.

2.3. Soil Metabolic Processes

Community process meaning synergistic associations between microorganisms defined Carbon and Nitrogen transformations. Those transformations are community process, nor a unique population process. Carbon and Nitrogen functional microorganisms' community assemblages were defined from a functional and dynamical perspective in the context of one agricultural management aspect that is the input or organic matter (Figure 1). For this reason, the Carbon consortium represents a group of microorganisms that synergistically converts cellulose into glucose considering that cellulose is one of the dominant molecules in green amendments or green soil covers. On the other hand, the input of Nitrogen, in terms of amendments is defined as organic Nitrogen, proteins and aminoacids that are mineralized by the second consortium that looks for represent a group of microorganisms that synergistically process organic Nitrogen and converts it to mineral Nitrogen; the system also considers the input of atmospheric Nitrogen.

2.4. Organic Carbon Transformation

The Carbon microbiota assemblage characteristics and attributes permits reflect the principal biological attributes (Table 1). The differentiation between the microorganisms that are part of the Carbon consortium is essentially functional, but the context is also physiological and biological. This specialized microbiota is

Table 1. Description of the carbon consortium.

Taxonomic unit	Microorganism name	Enzyme	Substrate	Product
Fungi	Fungus	Cellulase Exocellobiohydrolase	Cellulose	Cellodextrin Cellobiose
Bacteria/Archaea	Microbes	Betaglucosidase	Cellodextrin	Cellobiose
		Exocellulase		Cellobiose
Bacteria/Archaea	Bacteria	Alpha glucosidase	Cellobiose	Glucose
		6-fosfo-betaglucosidase	Cellobiose-6P	Glucose

defined by three different kinds of microorganisms considered as the simulation individuals or agents and these microorganisms populations has its own functional dynamics that allows that the community transform recalcitrant Carbon to produce labile available Carbon for all microorganisms and is expressed as the system glucose level. This Carbon metabolism is an emergent property of the system, resembling the interaction between the Carbon consortia, their exoenzymes and the sequential transformation of the cellulose to glucose (Table 1) in the form cellulose Cellodextrinàcellobioseàglucose (Figure 1) [23].

2.5. Nitrogen Transformation

Nitrogen consortium is proposed considering the different stages in the transformation of organic Nitrogen to mineral Nitrogen forms. This biotransformation has the potential to generate bioavailable Nitrogen for the plants in the form of nitrate. Additionally, N can enter the soil by the fixation process, that is developed by a specialized group of microorganisms called FixingBacts and resemble Nitrogen fixating bacteria (Table 2).

Ammonia and ammonium ions suffer a posterior transformation that is mediated by another group of microorganisms performing nitrification process. The microorganisms that perform the nitrification process are defined in Table 3.

2.6. Soil Microbiota Properties

Each group of agents or microorganism's populations are characterized by attributes (Table 4) that has been expressed as NetLogo variables associated to a specific biological function. Emergent community patterns arise from the possibility for each microorganism of take labile Carbon that in turn defines an internal energy level. The internal energy level defines the actions considered for microorganisms, movement, enzyme production, glucose assimilation, reproduction and mortality. Each action is characterized by one or more variables; the definition of this biological variables (Table 4) has been considered from the work of Moore [19].

Table 2. Description of the nitrogen consortium that develop the first stage of organic Nitrogen transformation.

Taxonomic Unit	Microorganism Name	Enzyme	Substrate	Product
Bacteria	Bacterias 2	Protease	Organic Nitrogen-proteins	L-asparagine
Fungi	Fungus 2	Protease	Organic Nitrogen-proteins	Arginine
Bacteria	Microbes 2	L-Asparaginase	Aminoacids. L-asparagine	Amonia NH_3
Bacteria	Microbes 3	Arginine-Deiminase	Aminoacids. Arginine	Ammonium NH_4
Nitrogen Fixing Bacteria	FixingBacts	Di Nitrogen oxidoreductase	Molecular Nitrogen N_2	Ammonia NH_3

Table 3. Consortium associated to nitrification process.

Taxonomic Unit	Microorganism Name	Enzyme	Substrate	Product
Nitrifying Bacteria	Nitrifs 1	Ammonia monooxygenase	Ammonia NH_3	Hydroxylamine NH_2OH
Nitrifying Bacteria	Nitrifs 2	Hydroxylamine oxidoreductase	Hydroxylamine	Nitrite NO_2
Nitrifying Bacteria	Nitrifs 3	Nitrite oxidoreductase	Nitrite	Nitrate NO_3

Table 4. Microbiota properties.

Action	Variables	Units	Description
Movement	Speed	Patches	Number of netlogo patches covered by a single microorganism
	Movement energetic cost	Energy units	Quantity of loss energy by movement by a single microorganism
Enzyme production	Threshold	Energy units	Patch enzyme level that defines if the microorganism produces the enzyme
	Enzyme production cost	Energy units	Quantity of energy loss energy by generation of an enzyme unit
	Energy buffer	Energy units	Minimum energy required for generation of an enzyme unit by a single microorganism

Continued

Energy uptake	Maximum assimilated glucose	Glucose per time unit	Maximum quantity of glucose particles that a single microorganism can uptake
	Glucose energy value	Energy units	Single microorganism quantity energy level increased by assimilated glucose unit
	Respired proportion	Proportion	Energy loss in the process of respiration
Reproduction	Energy to divide	Energy units	Internal single microorganism energy level required for cell division
Mortality	Contribution to cellulose	Particles	Quantity of particles that return to organic matter after microorganism death

2.7. Exoenzymes

The principal characteristic that differentiates each microorganism or agent group is functional. The exoenzyme produced by each microbial group implies specificity in terms of enzyme-substrate-product (Tables 1-3). This scheme allows representing the diversity in a way that rules out the functional redundancy as new works has expressed the "taxonomic distribution" of some enzymatic groups. A group of attributes that aim to reflect the biological reality of each represented microbial community from a functional perspective defines the regulation or control of the exoenzymes by the microbiota functional groups. Exoenzymes properties are defined by the duration of the enzyme in the soil, the diffusion rate of the enzyme, the number of product particles generated per unit of enzyme, the maximum enzyme speed, the number of particles of substrate that saturates the enzyme and the cost of producing an exoenzyme unit (Table 5).

2.8. Plant Growth

Considering the development of an agricultural system context, we defined the plant as an agent characterized by the uptake of energy based on the availability of nitrate, NO_3, in the soil. The values refer to an experiment developed in *BehaviorSpace*. Nitrite oxidoreductase cost is the energy that the microorganism that produces this specific enzyme loses per unit of enzyme generated. Maximum nitrate taken plants is the level of nitrate that the plant converts in new plant cells or plant growth, the nitrate energy value is the level of energy gained by the plant cells per unit of nitrate taken and the initial plants refers to the initial number of plant cells. These variables look for a representation of the variables that could affect plant growth in an agricultural system and the plant adaptation to its environment.

Table 5. Variables considered by the model for plant growth.

Nitrite oxidoreductase cost	Maximum nitrate taken by plant	Nitrate energy value to plant	Initial plant energy	Initial plant cells
1 energy unit	100 nitrate particles	1 energy unit	100 energy units	40 plant cells
100 energy units	1000 nitrate particles	100 energy units	1000 energy units	400 plant cells

2.9. Environment

In Netlogo, the environment consists of a grid composed of patches; these patches, represents for the system, soil fragments or plant root cells; the importance of the patches is that emulates different conditions in the proposed environment. In the case of this system, each grid is defined as a soil fragment, and characterized by the level of the enzymes and substrates including the glucose level. The microorganisms walk around the patches looking for glucose and the specific substrate in the metabolic scheme defined. The principal factors affecting soil microbiota that were considered in the subsequent simulations were the root exudates and conservation agriculture in terms of organic matter inputs (Table 6). The patches in this simulation could also take values of the plant root cells, which means that each root cell delivers labile Carbon for microorganisms' populations.

In addition to Table 5 and Table 6, the experiments designed in *Behavior-Space* of Netlogo, and the correspondent results presented in Figures 2-6, the community composition was not modified, presenting an initial number of 250 individuals for all of the runs. Considering the impotance of interspecific competition in an ecological context represented by the rhizosphere, the composition were maintained equal but other values observed (Table 5 and Table 6) were modified. So the composition patterns should be attributed to interspecific competition.

3. Results and Discussion

3.1. System Structure

The microbiota interacts in the agricultural system in terms of the input of organic matter, assuming that this practice allows microbiota development. This is represented as cellulose that is an abundant molecule and proteins, substrates processed by the microbiota (Figure 1).

3.2. System Dynamics and Simulations

Microbiota community dynamics

Eleven different populations (equivalent to agents in terms of IBM) have been proposed in the model, defining a community with two specialized consortia, one consortium for Carbon and the other for Nitrogen transformations. All the
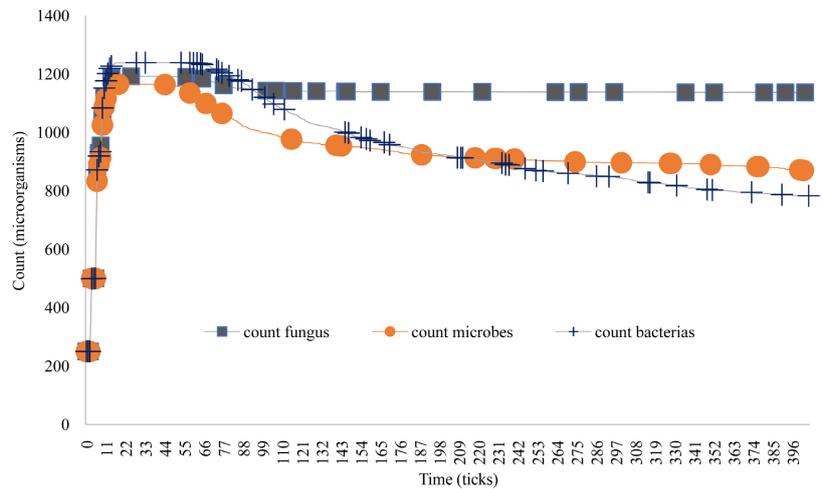
(a)

(b)

(c)
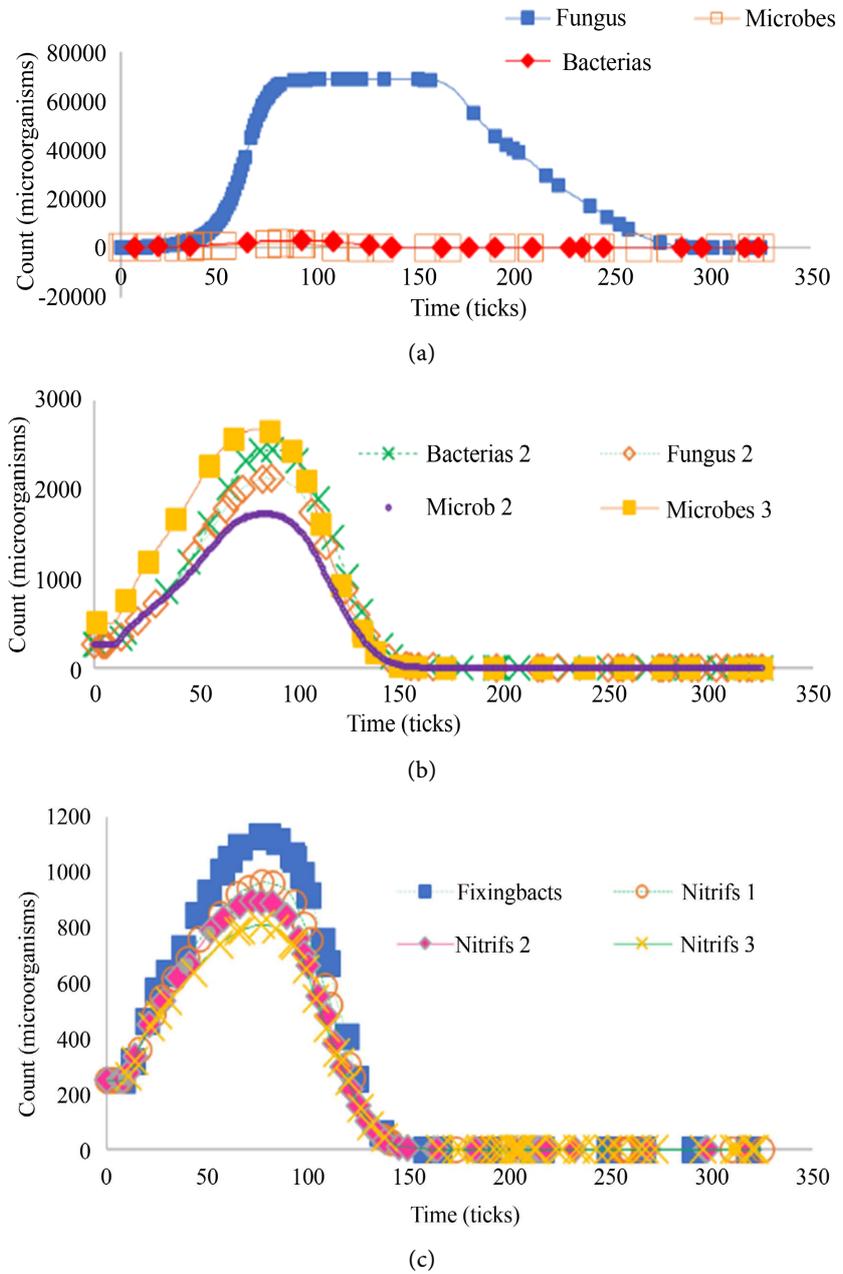
Figure 2. Microbiota growth patterns. (a)-(c) Growth under cellulose input.
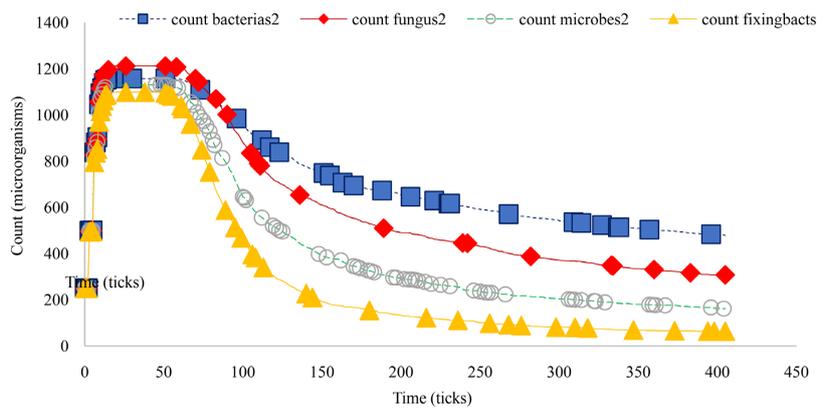
Table 6. Experiment considering the input of cellulose and glucose.

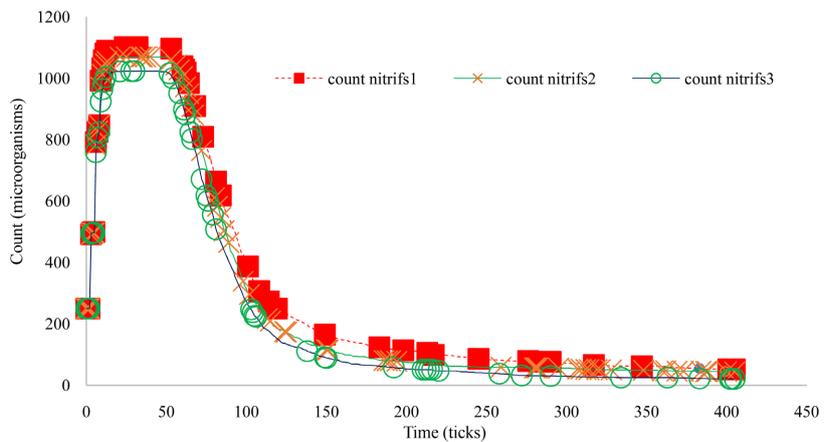| Initial cellulose	Glucose period	Glucose per tick	Cellulose per tick	Total root length
10 particles	1 - 5 - 20 ticks	100 particles	1000 particles	100 root cells
100 particles		350 particles	10000 particles	2000 root cells

populations that conform each consortium present its own dynamics, that altogether generates emergent patterns in terms of the abundance and hence the community structure. The population growth patterns suggests that the rhizospheric
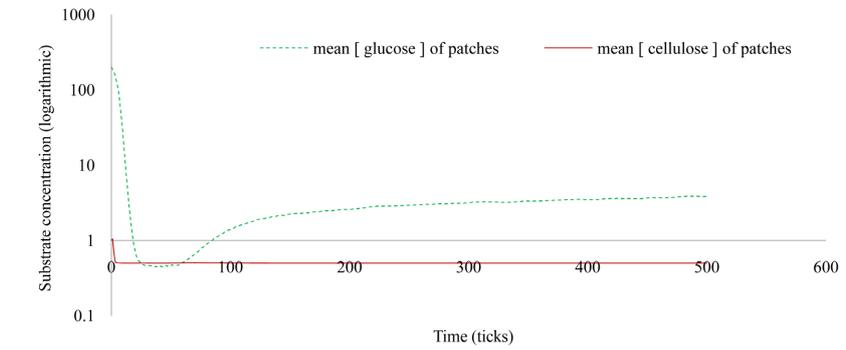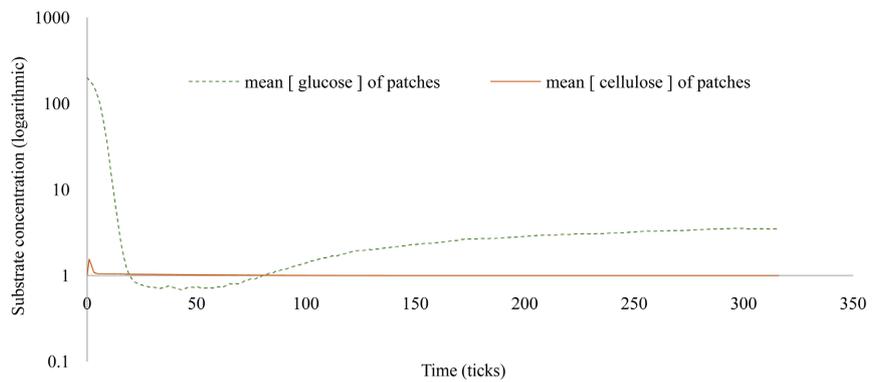
Figure 3. Microbiota growth patterns under cellulose and glucose input. (a) Carbon consortium. (b) and (c) Nitrogen consortium.
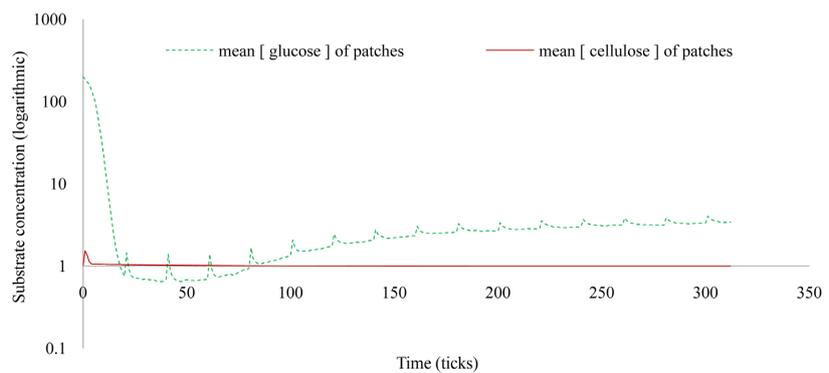
soil conditions allow the community sustainable growth and that the input of only cellulose (**Figures 2(a)-(c)**) is not enough to maintain microorganisms' populations.

(a)

(b)

(c)

Figure 4. Patterns of carbon substrates, cellulose and glucose behavior in the system, accordingly to variables presented in **Table 6**.

Some of the different populations that conforms the Carbon and Nitrogen consortia get a point of exponential growth but sharply declines, nonetheless under rhizospheric conditions (**Figures 3(a)-(c)**), meaning presence of roots in the simulation and glucose input from the roots, a slightly different tendency is observed.
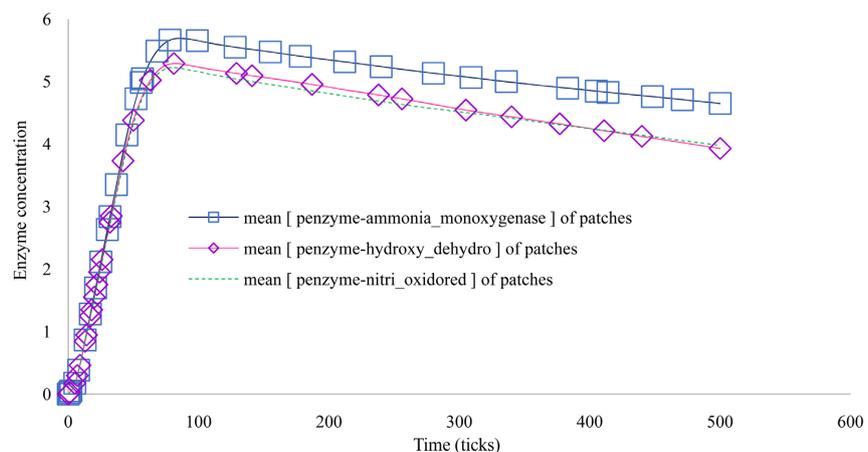
As the populations present differences, the model's population dynamics suggest that these results are in concordance with the rhizospheric environment theory, in which plant root supplies microbiota with nutrients, allowing defined groups to improve the fitness and hence the population patterns.
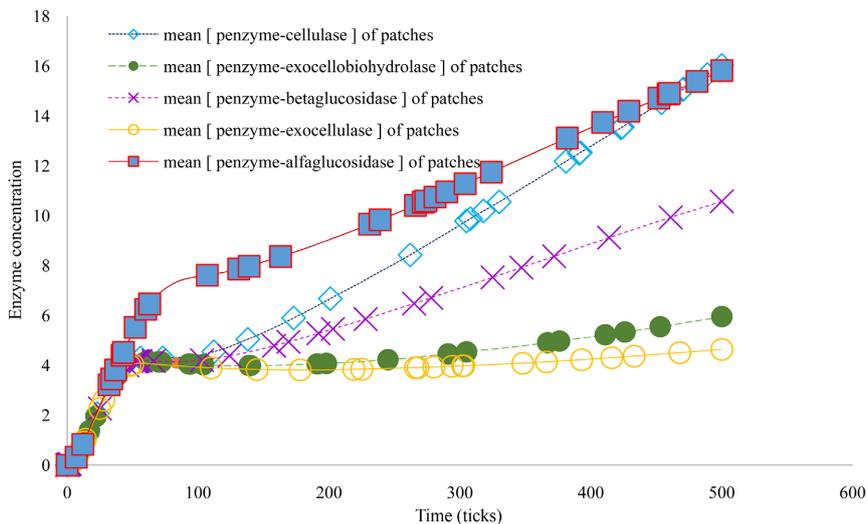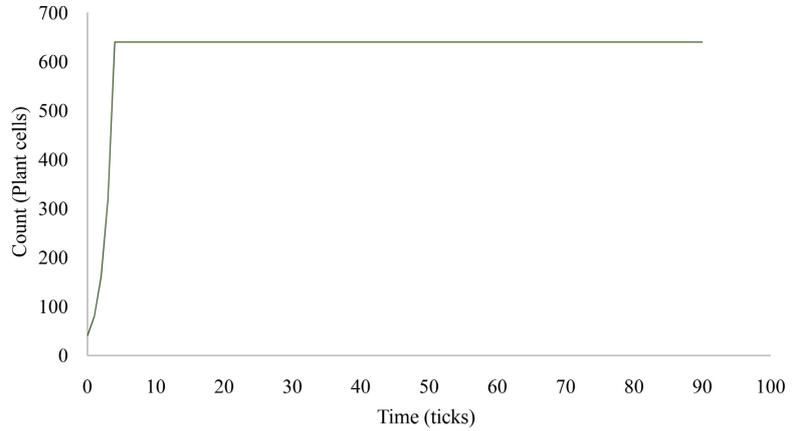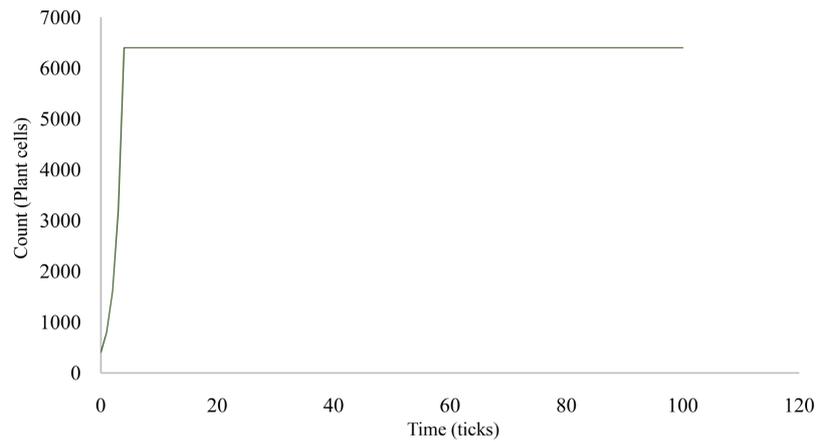
(a)
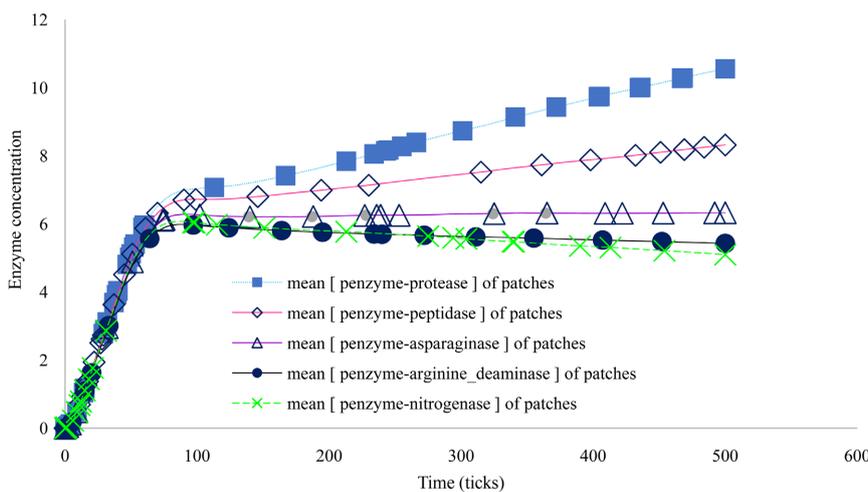
(b)

(c)

Figure 5. Exoenzymes in the system, under different glucose and cellulose input rates. (a) Exoenzymes correspondent to Carbon consortium. (b) and (c) Exoenzymes correspondent to Nitrogen consortium.

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(a)

(b)
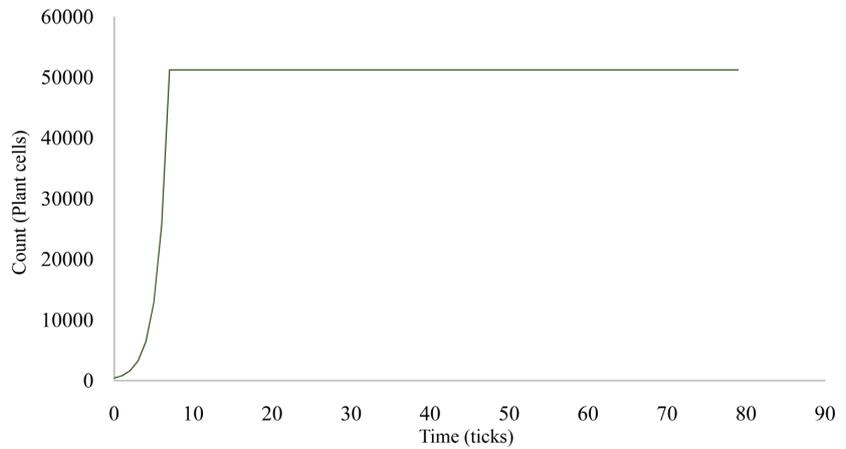
(c)

Figure 6. Plant growth considering different values for nitrate as energy source and initial vegetal cell numbers.

3.3. Rate of Substrates Input

To verify the behavior of the system organic Carbon substrates, cellulose and glucose, variation in the input rate of glucose and cellulose were developed along

with variation in the total root length of the rhizospheric soil. Varying the input of Carbon substrates cellulose and glucose, the number of cells defined as roots in the soil and the time between substrate inputs gives the substrate levels patterns (Table 6). The results suggest that the Carbon consortia processed the cellulose considering that this substrate doesn't accumulate in the system (Figure 4) and the microorganisms constantly use that glucose.

3.4. Exoenzymes

In the system simulation, enzymes could be considered as the principal response variables even over microorganism's population dynamics. The enzyme behavior in the system presents a different emergent pattern as the microorganism's dynamics (Figure 5), suggesting that the soil functional capacity depends on the microorganism population level but, depends too on other variables associated to the enzymes own identity. Variables considered in this sense are half time of the enzyme, the patch concentration of the enzymes, the biological cost of producing the enzyme for microorganisms, an energy level under which microorganisms doesn't produce the enzymes and the diffusion rate of the enzyme [19].

3.5. Plant Growth

The plant depends on the system nitrate generation for growth. At the same time, Nitrate is related to the process of cellulose metabolism to glucose for the microbiota energy and is related to the organic Nitrogen transformation proposed as input of proteins. Cellulose and organic Nitrogen are structural components of organic amendments, so the system considers conservative agricultural practices and its effect on the rhizospheric microbiota articulating ultimately to plant biomass generation. The variables used for plant definition (Table 5) defines different biomass production levels, nonetheless the nitrate generation is fundamental for the appearance of the observed patterns (Figure 6).

3.6. System Validation

The system was validated from the plant perspective in terms of vegetal growth patterns. These patterns indicate that in general, agricultural plants presents a lineal growth followed by a stationary phase, aspects that has been observed in *Chrysanthemun* [24] and other plants [25]. The growth patterns have been defining as expo linear growth, is recognized as a theoretical pattern in the context of agricultural plant development, and could be affirmed that the simulation (Figure 6) also shows this biomass increment.

4. Conclusion

This model could be considered as an innovative approach, in terms of elements integration and the context that was intended to represent; the explicit representation of microbiota, it's community structure and its functional capabilities

in an agricultural approach context; nonetheless, additional agricultural concepts should be considered in future developments that could be grounded or based on the present approach. Additionally, the presented model also has the capacity of represent structuring process in microbial assemblages in which inter and intra specific competition for the glucose is an important ecological element. This model should be considered as a frame that could be approached in posterior developments, from specific questions regarding specific plants and its core microbiota, meaning that this model could be defined as a "generic approach" and as a base for a new generation of models searching for, the explicit representation of microbial functional biodiversity.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Baveye, P.C., Berthelin, J. and Munch, J.C. (2016) Too Much or Not Enough: Reflection on Two Contrasting Perspectives on Soil Biodiversity. *Soil Biology and Biochemistry*, **103**, 320-326. https://doi.org/10.1016/j.soilbio.2016.09.008

[2] Louis, B.P., *et al.* (2016) Microbial Diversity Indexes Can Explain Soil Carbon Dynamics as a Function of Carbon Source. *PLoS ONE*, **11**, e0161251. https://doi.org/10.1371/journal.pone.0161251

[3] Maron, P.A., *et al.* (2018) High Microbial Diversity Promotes Soil Ecosystem Functioning. *Applied and Environmental Microbiology*, **84**, e02738-17.

[4] Berlemont, R. (2017) Distribution and Diversity of Enzymes for Polysaccharide Degradation in Fungi. *Scientific Reports*, **7**, Article No. 222. https://doi.org/10.1038/s41598-017-00258-w

[5] Berlemont, R. and Martiny, A.C. (2015) Genomic Potential for Polysaccharide Deconstruction in Bacteria. *Applied and Environmental Microbiology*, **81**, 1513-1519.

[6] Berlemont, R. and Martiny, A.C. (2016) Glycoside Hydrolases across Environmental Microbial Communities. *PLoS Computational Biology*, **12**, e1005300. https://doi.org/10.1371/journal.pcbi.1005300

[7] Hellweger, F.L., Clegg, R.J., Clark, J.R., Plugge, C.M. and Kreft, J.U. (2016) Advancing Microbial Sciences by Individual-Based Modelling S2 (Software Descriptions). *Nature Reviews Microbiology*, **2**, 2-4.

[8] Karimian, E. and Motamedian, E. (2020) ACBM: An Integrated Agent and Constraint Based Modeling Framework for Simulation of Microbial Communities. *Scientific Reports*, **10**, Article No. 8695. https://doi.org/10.1038/s41598-020-65659-w

[9] Quaghebeur, W. (2017) Individual-Based Modelling of Biodiversity in Microbial Communities. Master's Thesis, Universiteit Gent, Ghent.

[10] Prats, C., Ferrer, J., Gras, A. and Ginovart, M. (2010) Individual-Based Modelling and Simulation of Microbial Processes: Yeast Fermentation and Multi-Species Composting. *Mathematical and Computer Modelling of Dynamical Systems*, **16**, 489-510. https://doi.org/10.1080/13873954.2010.481809

[11] Portell, X., Pot, V., Garnier, P., Otten, W. and Baveye, P.C. (2018) Microscale Heterogeneity of the Spatial Distribution of Organic Matter Can Promote Bacterial Bio-

diversity in Soils: Insights from Computer Simulations. *Frontiers in Microbiology*, **9**, Article No. 1583. https://doi.org/10.3389/fmicb.2018.01583

[12] Ginovart, M. (2014) Discovering the Power of Individual-Based Modelling in Teaching and Learning: The Study of a Predator-Prey System. *Journal of Science Education and Technology*, **23**, 496-513. https://doi.org/10.1007/s10956-013-9480-6

[13] Leveau, J.H.J., Hellweger, F.L., Kreft, J.U., Prats, C. and Zhang, W. (2018) Editorial: The Individual Microbe: Single-Cell Analysis and Agent-Based Modelling. *Frontiers in Microbiology*, **9**, Article No. 2825. https://doi.org/10.3389/fmicb.2018.02825

[14] Juyal, A., *et al.* (2019) Combination of Techniques to Quantify the Distribution of Bacteria in Their Soil Microhabitats at Different Spatial Scales. *Geoderma*, **334**, 165-174. https://doi.org/10.1016/j.geoderma.2018.07.031

[15] Jiao, S., Xu, Y., Zhang, J., Hao, X. and Lu, Y. (2019) Core Microbiota in Agricultural Soils and Their Potential Associations with Nutrient Cycling. *mSystems*, **4**, e00313-18.

[16] Bodine, E.N., Panoff, R.M., Voit, E.O. and Weisstein, A.E. (2020) Agent-Based Modeling and Simulation in Mathematics and Biology Education. *Bulletin of Mathematical Biology*, **82**, Article No. 101. https://doi.org/10.1007/s11538-020-00778-z

[17] Banitz, T., Gras, A. and Ginovart, M. (2015) Individual-Based Modeling of Soil Organic Matter in NetLogo: Transparent, User-Friendly, and Open. *Environmental Modelling & Software*, **71**, 39-45. https://doi.org/10.1016/j.envsoft.2015.05.007

[18] Resat, H., Bailey, V., McCue, L.A. and Konopka, A. (2012) Modeling Microbial Dynamics in Heterogeneous Environments: Growth on Soil Carbon Sources. *Microbial Ecology*, **63**, 883-897. https://doi.org/10.1007/s00248-011-9965-x

[19] Moore, J.C., Boone, R.B., Koyama, A. and Holfelder, K. (2014) Enzymatic and Detrital Influences on the Structure, Function, and Dynamics of Spatially-Explicit Model Ecosystems. *Biogeochemistry*, **117**, 205-227.
https://doi.org/10.1007/s10533-013-9932-3

[20] Yi, X., Yi, K., Fang, K., Gao, H., Dai, W. and Cao, L. (2019) Microbial Community Structures and Important Associations Between Soil Nutrients and the Responses of Specific Taxa to Rice-Frog Cultivation. *Frontiers in Microbiology*, **10**, Article No. 1752. https://doi.org/10.3389/fmicb.2019.01752

[21] Louis, B.P., Maron, P.A., Viaud, V., Leterme, P. and Menasseri-Aubry, S. (2016) Soil C and N Models that Integrate Microbial Diversity. *Environmental Chemistry Letters*, **14**, 331-344. https://doi.org/10.1007/s10311-016-0571-5

[22] Tong, L., Zhu, L., Lv, Y., Zhu, K., Liu, X. and Zhao, R. (2019) Response of Organic Carbon Fractions and Microbial Community Composition of Soil Aggregates to Long-Term Fertilizations in an Intensive Greenhouse System. *Journal of Soils and Sediments*, **20**, 641-652. https://doi.org/10.1007/s11368-019-02436-x

[23] Choi, J., *et al.* (2018) Spatial Structuring of Cellulase Gene Abundance and Activity in Soil. *Frontiers in Environmental Science*, **6**, Article No. 107.
https://doi.org/10.3389/fenvs.2018.00107

[24] Jeong, H.L., Goudriaan, J. and Challa, H. (2003) Using the Expolinear Growth Equation for Modelling Crop Growth in Year-Round Cut Chrysanthemum. *Annals of Botany*, **92**, 697-708. https://doi.org/10.1093/aob/mcg195

[25] Martin, T.N., Neto, D.D., Vieira, P.A., Pereira, A.R., Manfron, P.A. and Christoffoleti, P.J. (2012) Modelo modificado de estimação da produtividade deplecionada e potencial da soja. *Acta Scientiarum-Agronomy*, **34**, 369-378.