University of São Paulo
"Luiz de Queiroz" College of Agriculture

Soil carbon and nitrogen dynamics as affected by crop diversification and nitrogen fertilization under grain production systems in the Cerrado region

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Thesis presented to obtain the degree of Doctor in Science. Area: Soil and Plant Nutrition

Piracicaba
2021

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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2021

Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Sattolo, Thales Meinl Schmiedt

Soil carbon and nitrogen dynamics as affected by crop diversification and nitrogen fertilization under grain production systems in the Cerrado region / Thales Meinl Schmiedt Sattolo. - - versão revisada de acordo com a resolução CoPGr 6018 de 2011. - - Piracicaba, 2021.

133 p.

Tese (Doutorado) - - USP / Escola Superior de Agricultura “Luiz de Queiroz”.

1. Estrutura do solo 2. Estoque de nutrientes 3. Dinâmica do carbono 4. ^{14}C-substratos I. Título

ACKNOWLEDGMENTS

First, I am remarkably grateful to the Universe and the spirituality. No more words could express my feelings.

I really thank my body and spirit for carrying myself towards moral development despite of every external reverse energy.

I am deeply thankful to my parents Nivaldo Sattolo and Maria Sueli M. S. Sattolo, my sister Natalia M. S. Sattolo and my girl Tasiane de O. Ribeiro for encouraging and supporting my decisions. You have been teaching me to progress as human being. I love you all!

People are self-connected to each other in a unique manner and from their relationship we evolute regardless of the responses. Here, I apologize for every inconvenient behavior to others. Likewise, I specially thank to friends, colleagues, roommates, interns, co-workers, workers, technicians, professors, supervisors, partners, reviewers, leaders, and everyone who has been sharing their experiences to help on my journey as a researcher.… Even you who read it now! Thank you all!

I really appreciate 2018 and 2019 for the most intense years ever in my life.

I thank the following institutions for distinct and significant contribution to develop this tiny piece of polish information known as Thesis:

- my place “Luiz de Queiroz” College of Agriculture - University of São Paulo (ESALQ/USP), the employees, professors and supervisors for all knowledge and opportunities.
- my home Covil and brothers for the great moments of fraternity and joy...Cheers!
- the São Paulo Research Foundation (FAPESP) for providing my scholarships as doctorate student (grant # 2017/20602-6) and intern research abroad (grant # 2018/24376-3).
- the Bangor University, the Fundação Agrisus, the Fundação de Apoio à Pesquisa Agropecuária de Mato Grosso (FMT) and the International Plant Nutrition Institute (IPNI).

EPIGRAPH

To reach a researcher status (as PhD degree) is not a matter of – hard – studying and full-time dedication to science but it's a long-term process of self-transformation against the infinite human ignorance, learning to fail and to struggle towards the modesty. By accepting my absolute ignorance, facing the universe "laws" was the first step: I'm nothing but star dust being recycled in an alive body. To recognize my complete insignificance along human history still as the second step: I'm nobody but one over billions of others ever existed. Next step? I don't know further! But I've been recognizing something that may indicate next: a better science is made of creativity and assistance instead of nonsense academic/research policies.

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RESUMO

Dinâmica do carbono e nitrogênio do solo afetada pela diversificação de culturas e fertilização nitrogenada em sistemas de produção de grãos na região do Cerrado

A agricultura tem sido amplamente responsável pelas emissões de C dos solos, principalmente por meio da mudança de uso da terra (LUC) de vegetação nativa (NV) para agrossistemas. Apesar disso, o Brasil é referência em ciência e tecnologia na agricultura com diretrizes políticas de sustentabilidade e segurança alimentar. O melhor exemplo de desenvolvimento recente da agricultura brasileira foi a transformação dos ecossistemas do Cerrado, convertendo-se de área agrícola marginal para um grande celeiro global devido às práticas de manejo avançadas, como plantio direto, sucessão e rotação de culturas, fertilização adequada e maquinário de alto rendimento. Neste cenário de mudança de uso do solo (+ emissões de C) e adoção de agricultura conservacionista de alta tecnologia (- emissões de C) no Cerrado, experimentos de longo prazo foram estudados para: i) avaliação da estrutura do solo através de análises laboratoriais e *visual evaluation os soil structure* (VESS); ii) quantificação dos estoques de frações orgânicas e inorgânicas de C e N em profundidade no solo, e iii) compreensão da dinâmica do C por meio da modelagem da cinética de decomposição de ^{14}C-substratos. A avaliação da estrutura do solo revelou que a LUC do Cerrado para os sistemas de produção de soja e milho (SMPS) afetou negativamente a qualidade estrutural do solo e suas propriedades físicas, independentemente da prática de manejo e camada do solo. Um ligeiro declínio na qualidade física do solo foi detectado (0-0,2 m) no SMPS, relacionado às operações de maquinário necessárias para manejar tratamentos sob rotação/sucessão de cultura. O VESS identificou com sucesso as mudanças na estrutura do solo induzidas pelo uso e manejo do solo, enquanto análises laboratoriais detectaram mudanças em funções específicas associadas à porosidade e dinâmica da água. A quantificação de C e N no solo mostrou que a conversão de NV para SMPS levou à diminuição desses estoques no solo acentuado na camada de 0-0,2 m para estoques totais e até 1,0 m para estoques dissolvidos. Embora não houve diferenças no C e N do solo para os estoques totais entre os tratamentos de SMPS, o ensaio de mineralização de longo prazo indica que os efeitos poderão ser verificados no experimento de campo no longo prazo. A dinâmica de decomposição de C variou principalmente dentro dos fatores *camadas*, *substratos* e *priming*, e menos em *local*, *agroecossistemas* e *disponibilidade de nutrientes*. Em média, maior eficiência de uso de C (CUE) foi encontrada sob SMPS, amostras de subsolo e aplicação de celulose, como resposta da comunidade microbiana do solo. O *priming* demonstrou que a *lag-phase* inicial na cinética de decomposição do subsolo estava provavelmente relacionada a microrganismos dormentes em vez de reduzida biomassa microbiana e baixa disponibilidade de nutrientes em Latossolos. Em geral, a conversão de NV para SMPS promove a depleção das funções do solo (i.e. estrutura física, estoques e ciclagem de nutrientes). Por outro lado, a sucessão soja-milho é um sistema de produção de grãos bem-sucedido, proporcionando duas colheitas a cada ano agrícola enquanto mantém os estoques de C e N do solo adequados ao SMPS. Em última análise, o SMPS no Cerrado tem grande potencial para aumentar a estabilização de C principalmente no subsolo.

Palavras-chave: Estrutura do solo, Estoque de nutrientes, Dinâmica do carbono, ^{14}C-substratos

ABSTRACT

Soil carbon and nitrogen dynamics as affected by crop diversification and nitrogen fertilization under grain production systems in the Cerrado region

The agriculture has been largely responsible for soils C emissions mainly through land use change (LUC) from native vegetation (NV) to agrosystem. Despite that, Brazil is reference on soil (and crop) science and technology in agriculture towards sustainability and food security policies. The best example of the recent development of Brazilian agriculture was the transformation of Cerrado ecosystems from a *non-fit agricultural land* to a current *major global breadbasket* due to advanced management practices such as no-tillage, double cropping, proper fertilization and high-performance machinery. In this scenario of land use change (+ C emissions) and adoption of high-tech conservation agriculture (- C emissions) in Cerrado that long-term experiments were evaluated for: i) soil structure assessment through laboratory analyses and the visual evaluation of soil structure (VESS); ii) nutrient storage quantification through the soil C and N pools at depth, and iii) C dynamics understanding through modelling the decomposition kinetics of ^{14}C-labelled substrates. The soil structure assessment reveled that the LUC from Cerrado to soybean and maize production systems (SMPS) negatively affected the soil structural quality and the physical properties, regardless of management practice and soil layer. Also, a slight decline in soil physical quality was detected (0-0.2 m) in SMPS related to the machinery operations required to manage a more diverse crop sequence. The VESS approach successfully identified changes in the soil structure induced by the soil use and management whereas laboratory analyses detected changes in specific functions associated to porosity and water dynamic. The quantification of soil C and N storage showed that the conversion from NV to SMPS lead to a soil C and N depletion stressed at 0-0.2 m layer for total stocks and down to 1.0 m for dissolved stocks. Although we had no differences on soil C and N for total stocks between SMPS treatments, the long-term mineralization assay indicates that the effects might be evident on field experiment further up. The C decomposition dynamics varied mostly within *layers*, *substrates* and *priming* than *site*, *agroecosystems,* and *nutrient availability*. On average, higher C use efficiency (CUE) were found under SMPS, subsoils samples and cellulose application as response of soil microbial community. *Priming* demonstrated that the initial lag-phase on decomposition kinetics of subsoils were probably related to dormant microorganisms instead of minor microbial biomass and low nutrient availability in Oxisols. Overall, the conversion from NV to SMPS promotes depletion of soil functions (i.e., physical structure, stocks, and nutrient cycling). On the other hand, the soybean-maize succession is a successful grain production system providing two harvesting every year while holding the soil C and N stocks suitable for SMPS. Ultimately, the SMPS in Cerrado have great potential for C stabilization mostly in subsoil.

Keywords: Soil structure, Nutrient stocks, Carbon dynamics, ^{14}C-labelled substrates

1 INTRODUCTION

Soils under agrosystems have been suffering a progressive loss of organic matter, leading to a decline in soil quality, release of damaging greenhouse gases, and the sub-optimal delivery of many ecosystem services. Despite that, the adoption of conservation agricultural practices worldwide have been attempting to turn over the adverse process of soil degradation or at least mitigate it while holding the crop yield. In Brazil, management practices like no-till, double cropping, crop rotation and proper fertilization have been addressed to grain production system resulting in intensified agrosystems with increased yield potential while maintaining soil functions (McDaniel et al., 2014; Moraes Sá et al., 2015; Salton et al., 2014).

In fact, comparison of management practices on agrosystems still in context due to its mixed effects found in many studies (i.e., positive, negative, or null). For instance, the effect of crop rotation and fertilization may increase nutrient cycling and impacting soil nutrient stocks but their effects on soil physical quality is non-conclusive (Batlle-Bayer et al., 2010; Kauer et al., 2015; McDaniel et al., 2014; Riggs et al., 2015). Even no-till, considered a suitable practice for soil C stabilization and enhancing C stocks, have been recently brought up back in context due to evidences supporting plowing soil residues in depth to promote a conservative environment in subsoil for C storage through water saturation, low aeration and slow microbial activity (Alcántara et al., 2017, 2016). Nevertheless, the identification of soil physicochemical responses on soil functions, mainly on soil structure and nutrient cycling and storage, as affected by such management practices has been largely assessed at long-term field experiments in grain producing regions in Brazil (Calegari et al., 2008; da Silva et al., 2004; Fabrizzi et al., 2009; Ferreira et al., 2013; Moraes Sá et al., 2015; Salton et al., 2014; Vieira et al., 2009). Likewise, this thesis evaluated the effects of agrosystems under different management practices in a nine-years field experiment in Cerrado. It was split into three chapters approaching specific soil functions as follows:

- Chapter 2 for soil structure assessment through laboratory analyses and the visual evaluation of soil structure;
- Chapter 3 for nutrient storage quantification through the soil C and N pools at depth;
- Chapter 4 for nutrient cycling understanding through modelling the decomposition kinetics of ^{14}C-labelled substrates.

References

Alcántara, V., Don, A., Vesterdal, L., Well, R., Nieder, R., 2017. Stability of buried carbon in deep-ploughed forest and cropland soils - Implications for carbon stocks. Sci. Rep. 7, 1–12. https://doi.org/10.1038/s41598-017-05501-y

Alcántara, V., Don, A., Well, R., Nieder, R., 2016. Deep ploughing increases agricultural soil organic matter stocks. Glob. Chang. Biol. 22, 2939–2956. https://doi.org/10.1111/gcb.13289

Batlle-Bayer, L., Batjes, N.H., Bindraban, P.S., 2010. Changes in organic carbon stocks upon land use conversion in the Brazilian Cerrado: A review. Agric. Ecosyst. Environ. 137, 47–58. https://doi.org/10.1016/j.agee.2010.02.003

Calegari, A., Hargrove, W.L., Rheinheimer, D.D.S., Ralisch, R., Tessier, D., De Tourdonnet, S., Guimarães, M.D.F., 2008. Impact of long-term no-tillage and cropping system management on soil organic carbon in an oxisol: A model for sustainability. Agron. J. 100, 1013–1019. https://doi.org/10.2134/agronj2007.0121

da Silva, J.E., Resck, D.V.. S., Corazza, E.J., Vivaldi, L., 2004. Carbon storage in clayey Oxisol cultivated pastures in the “Cerrado” region, Brazil. Agric. Ecosyst. Environ. 103, 357–363. https://doi.org/10.1016/j.agee.2003.12.007

Fabrizzi, K.P., Rice, C.W., Amado, T.J.C., Fiorin, J., Barbagelata, P., Melchiori, R., 2009. Protection of soil organic C and N in temperate and tropical soils: Effect of native and agroecosystems. Biogeochemistry 92, 129–143. https://doi.org/10.1007/s10533-008-9261-0

Ferreira, A.O., Amado, T.J.C., Nicoloso, R.S., Sá, J.C.M., Fiorin, J.E., Hansel, D.S.S., Menefee, D., 2013. Soil carbon stratification affected by long-term tillage and cropping systems in southern Brazil. Soil Tillage Res. 133, 65–74. https://doi.org/10.1016/j.still.2013.05.011

Kauer, K., Tein, B., Sanchez de Cima, D., Talgre, L., Eremeev, V., Loit, E., Luik, A., 2015. Soil carbon dynamics estimation and dependence on farming system in a temperate climate. Soil Tillage Res. 154, 53–63. https://doi.org/10.1016/j.still.2015.06.010

McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? a meta-analysis. Ecol. Appl. 24, 560–570. https://doi.org/10.1890/13-0616.1

Moraes Sá, J.C., Séguy, L., Tivet, F., Lal, R., Bouzinac, S., Borszowskei, P.R., Briedis, C., dos Santos, J.B., da Cruz Hartman, D., Bertoloni, C.G., Rosa, J., Friedrich, T., 2015. Carbon depletion by plowing and its restoration by no-till cropping systems in oxisols of subtropical and tropical agro-ecoregions in Brazil. L. Degrad. Dev. 26, 531–543. https://doi.org/10.1002/ldr.2218

Riggs, C.E., Hobbie, S.E., Bach, E.M., Hofmockel, K.S., Kazanski, C.E., 2015. Nitrogen addition changes grassland soil organic matter decomposition. Biogeochemistry 125, 203–219. https://doi.org/10.1007/s10533-015-0123-2

Salton, J.C., Mercante, F.M., Tomazi, M., Zanatta, J.A., Concenço, G., Silva, W.M., Retore, M., 2014. Integrated crop-livestock system in tropical Brazil: Toward a sustainable production system. Agric. Ecosyst. Environ. 190, 70–79. https://doi.org/10.1016/j.agee.2013.09.023

Vieira, F.C.B., Bayer, C., Zanatta, J.A., Mielniczuk, J., Six, J., 2009. Building up organic matter in a subtropical paleudult under legume cover-crop-based rotations. Soil Sci. Soc. Am. J. 73, 1699. https://doi.org/10.2136/sssaj2008.0241

2 EFFECTS OF SOIL TILLAGE AND CROP DIVERSIFICATION ON SOIL PHYSICAL QUALITY IN BRAZILIAN CERRADO

Abstract

The Cerrado region covers 204 M ha of Brazil and its currently considered the major global breadbasket. Conservation management practices, such as no-tillage and crop rotation are widespread through Cerrado to sustain soil quality and increase crop yield. However, the effects of such practices on soil physical quality is still a challenge for the local farmers as well as the search for on-farm methods to identify the impacts on soil structure. Therefore, we evaluated the effects of management practices (i.e., tillage, crop diversity) in two long-term experiments (+9 years) on soil physical quality through laboratory analyses and the visual evaluation of soil structure (VESS) at 0-0.1 and 0.1-0.2 m layers. The treatments involved soybean/fallow under conventional tillage (SF), soybean/fallow under no-till (SN), soybean/maize succession under no-till (SM), crop rotation under no-till including soybean, maize, grasses, and legumes (CR) and an additional representative native vegetation (NV). The land transition from Cerrado (NV) to agricultural cropping systems negatively affected the soil structural quality and the physical parameters, regardless of management practice and soil layer. The soil bulk density and penetration resistance showed no differences among management practices. However, SN presented better porosity-associated soil parameters (i.e., macroporosity, aeration capacity and water storage retention) and water-associated soil parameters (i.e., hydraulic conductivity) than SM and CR relating differences in subsurface between crop diversity. This result suggests a slight decline in soil physical quality related to the machinery operations frequency required to manage a more diverse crop sequence in alignment to the soil resilience promoted by fallow. Ultimately, the VESS approach successfully identified changes in the soil structure induced by the soil use whereas laboratory analyses detected changes in specific functions associated to porosity and water dynamic by soil management. Long-term soil physical monitoring is fundamental to design more sustainable diversified cropping system in Brazilian Cerrado.

Keywords: No tillage, Crop rotation, Crop sucession, Visual Evaluation of Soil Structure (VESS)

2.1 Introduction

Conservation agriculture (CA) has been considered as a promising alternative to sustain soil health, increase crop yield, reduce greenhouse gas emissions and consequently achieve food security and mitigate global climate changes (Foley et al., 2011; Kassam et al., 2015; Sá et al., 2017). Hence, CA adoption spread remarkably worldwide over cropland area in the last years by increasing 70% from 2009 to 2016. In Brazil, CA adoption has been diffusing mainly over degraded pasturelands and covers more than 32 million ha (Kassam et al., 2019).

The CA is based on the principles of minimum mechanical soil disturbance (no-till), maintenance of crop residues on soil surface, diversification of crop species and integrated nutrient management (Lal, 2015; Xue et al., 2015). Absence of soil disturbance reduce the rate of crop residue decomposition and indirectly influence the soil structure through the continuous contribution of carbon to formation and stabilization of soil aggregates (Ferreira et al., 2018). On the other hand, tillage system promotes soil aeration and aggregates disruption resulting in an interruption of their genesis, decrease of their stability and acceleration of carbon losses to atmosphere (Six et al., 2004). Globally, no-till soils have higher loading capacity, greater infiltration and plant-available water and enhanced soil structural quality (Blanco-Canqui and Ruis, 2018).

Nevertheless, there are some challenges for CA adoption in a long-term since heavy machinery traffic has intensified soil compaction (Keller et al., 2019) by increasing soil bulk density and resistance to root penetration in the surface layers, especially in clayey soils (Cavalieri et al., 2009). In this context, crop diversification has a fundamental role recovering the structural quality of soils under CA through cropping (in rotation) plants with deep root system, which may mitigate soil compaction effects to plant growth due to the formation of deep and stable biopores (Guedes Filho et al., 2013; Han et al., 2015; Landl et al., 2019). Biopores can even drive the root development of subsequent crop (Han et al., 2016). Crop diversification affects the amount and quality of the residues added in the soil, increasing biochemical interactions between plants and soil microorganisms (Pires et al., 2020; Reich et al., 2012a; Venter et al., 2016), alleviating soil compaction and enhancing soil structure and water infiltration (Anghinoni et al., 2019; Blanco-Canqui et al., 2015).

Soil structure drives critical processes and functions in the environment (Bronick and Lal, 2005; Rabot et al., 2018). Therefore, several soil parameters have been used as indicator to evaluate shape, stability and functionally of soil structure e.g. bulk density, porosity, water storage capacity, soil strength, hydraulic conductivity (Bünemann et al., 2018; Rabot et al., 2018). More recently, visual methods has been developed to provide a simple, but reliable evaluation of soil structure quality directly in the field (Ball et al., 2017, 2007; Emmet-Booth et al., 2016). One of these method, the Visual Evaluation of Soil Structure (VESS), developed by Ball et al. (2007) and improved by Guimarães et al. (2011), is a simple, quick and on-farm easy-to-understand test that allows the evaluation of soil structural quality based on a semi-

quantitative and integrative approach. Visual evaluations are complementary to the quantitative laboratory methods traditionally used for assessing soil structure changes (Ball et al., 2017, 2007). The VESS scores can also provide a first approximation of overall soil quality status (Cherubin et al., 2016b). The VESS method has been applied in different regions of world, encompassing contrasting soil types, management practices and land uses. In Brazil, several studies had applied the VESS method (Franco et al., 2019) from lowland soils in the south (Tuchtenhagen et al., 2018) to cohesive soils in northeast (Cavalcanti et al., 2020) and Amazon soil in the north (Guimarães et al., 2017); however, there is a lack of studies focused on evaluating soil structure changes induced by CA in Cerrado, the main grain-producing region of the country.

Therefore, the hypotheses tested in this study were: (i) compared to native vegetation, soils under agricultural use have their physical quality degraded; however, adoption of CA systems, including no-tillage and crop rotation (high crop diversity), are suitable strategies to improve soil structure and mitigate soil physical degradation; (ii) soil structure changes induced by CA systems can be properly detected by the VESS method. To test the hypotheses, we evaluated field experiments over a gradient of CA treatments in Brazilian savanna (Cerrado biome) aimed to investigate the long-term effects of crop diversity and soil management on soil structure through applying both field visual evaluation (i.e., VESS method) and traditional laboratory analyses.

2.2 Material and methods

2.2.1 Site description and experimental design

The study was carried out at the Experimental Station of Cachoeira (ESC) of Fundação MT located in Itiquira, Mato Grosso state (17°9'18,44"S, 54°45'15,32"W and 490 m; Fig. 1), central-western Brazil. This region is covered by the Cerrado biome (Brazilian savanna), representing an important region of agricultural expansion in Brazil. The climate is classified as tropical wet-dry climate, Aw type accordingly to Köppen-Geiger’s classification, which is characterized by rainfall concentrated in the spring and summer (October to April) while the dry season occurs in the autumn and winter (May to September) (Alvares et al., 2013). The mean annual temperature is 24.5 °C and the annual precipitation is 1,260 mm (last 10-yr average).

In the study site, the conversion from wooded savanna (Cerrado) to pasture occurred in the 1970s. However, it was not possible to identify the exact year of this

land-use change. In 1992, pastures were converted to soybean or maize monocropping under annual tillage. A few years later, the no-tillage system was adopted and then in 2006 to soybean/maize succession under no-till from which has been received high-technology inputs (e.g., lime, phosphogypsum, P fertilizers) for high grain yields. In 2008, the ESC started establishing experiments under different grain production systems and two of them (~0.3 km apart each other) were chosen for this study. The experiment 1 (E1) evaluated three agrosystems involving crop succession and crop rotation with different N rates for maize production, while the experiment 2 (E2) evaluated eight agrosystems involving monocropping, crop succession and crop rotation for soybean production. The experimental design at both experiments was a randomized complete block with four replications. The experimental plots were sized as 892 and 600 m^2 for E1 and E2, respectively. Both experiments are located within the same soil type and distant < 200 m from each other in a flat landscape.

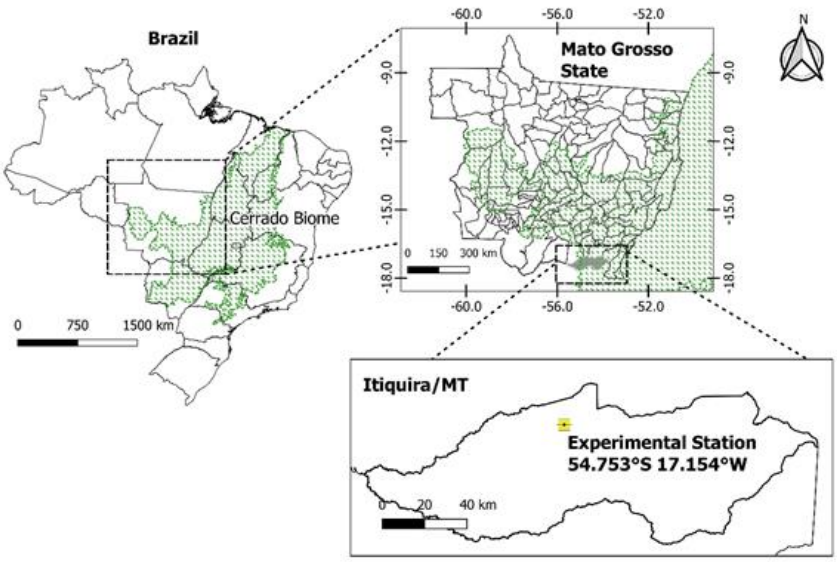


Fig. 1. Location of experimental station in Itiquira, Mato Grosso state, central Brazil

Among those treatments, we selected the following treatments to evaluate the effects of CA systems on soil structure: SF: soybean/fallow under conventional tillage (annual heavy-disc harrowing) from E2; SN: soybean/fallow under no-till from E2; SM: soybean/maize succession under no-till from E1; and CR: crop rotation under no-till

including soybean, maize, grass and legumes in rotation from E1 (Fig. 2). The above sequence of treatments represents a gradient of CA from low to high conservation. The installing of both experiments involved just the soil tillage with chisel plowing and heavy-disc harrowing up to 0.40 m depth. All crops were mechanically sown and harvested over the experiments while further mechanical operations (i.e., spraying application) were run aside them. So, the frequency in machinery operations were proportional to the number of crops involved in each agrosystem, except for *Urochloa sp.* under CR that were sown together with maize and chemically desiccated in the end-season. After +9 years experiencing the treatments, the agrosystems cumulated 27, 18, 35 and 29 operations for SF, SN, SM and CR, respectively. Crop residues were deposited on the soil surface during harvesting. In addition, a representative native vegetation (NV) of undisturbed wooded Cerrado located near of experimental sites (~4 km) was used as a reference area and four pseudo-replications (~600 m^2-size) were delimited ~50 m apart each other.

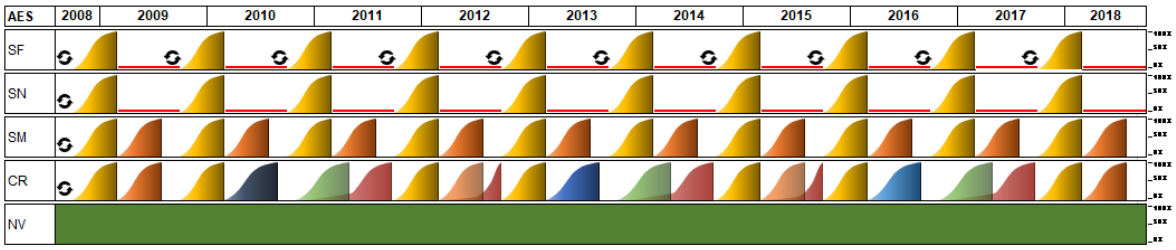


Fig. 2. Timeline of management practices and cultivation in each cropping system. AES: agroecosystems; SF: soybean/fallow under conventional tillage; SN: soybean/fallow under no-till; SM: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; NV: native vegetation. , soybean growth cycle sown in October and harvested in February; , maize growth cycle sown in March and harvested in July; , maize growth cycle sown in November and harvested in April; , *Urochloa ruziziensis* growth cycle sown in November and desiccated in September; , *Urochloa ruziziensis* growth cycle sown in March and desiccated in July; , *Crotalaria spectabilis* growth cycle sown in March and desiccated in July; , *Crotalaria ochroleuca* growth cycle sown in March and desiccated in July; , *Cajanus cajan* growth cycle sown in March and desiccated in September; , undisturbed savannah wooded; , heavy-disc harrowing; — fallow period. The values on the right side are percentage of the biomass accumulation by crop

The soils were classified as clayey Typic Haplustox (Soil Survey Staff, 2014), typical from Cerrado biome, which is highly weathered, found a predominance of the 1:1 clay mineral (kaolinite), iron- (goethite and hematite) and aluminum-oxide (gibbsite)

in the clay-size fraction (Coelho, 2019). The soil characterization of the treatments showed similar soil texture, cation exchange capacity and moisture at sampling among agrosystems and NV (Table 1).

Table 1. Physical and chemical soil characteristics from the 0 to 0.2 m depth from agrosystems under gradient of CA and in a representative undisturbed native vegetation

Treat.	pH	TOC	CEC	θ_f	Sand	Silt	Clay
	H_2O	g kg^{-1}	$mmol_c$ kg^{-1}	g g^{-1}	g kg^{-1}		
0-0.1 m							
SF	5.4	24.9	111.2	0.30	266	28	706
SN	5.4	26.6	115.8	0.27	255	34	711
SM	5.7	24.6	125.3	0.23	268	31	701
CR	5.8	31.5	124.1	0.22	261	27	712
NV	4.4	43.8	122.1	0.27	254	43	703
0.1-0.2 m							
SF	5.4	22.3	98.7	0.25	260	34	706
SN	5.3	21.7	100.4	0.23	258	25	717
SM	5.7	21.7	100.8	0.21	268	39	693
CR	5.7	22.5	101.2	0.22	260	29	711
NV	4.9	31.4	113.9	0.25	251	27	722

All soil characteristics were analyzed as van Raij et al. (2001). TOC: total organic carbon; CEC: cation exchange capacity at pH 7.0; θ_f: field soil moisture at sampling.

2.2.2 Visual Evaluation of Soil Structure (VESS) measurements and soil sampling

The VESS measurements were performed as described by Guimarães et al. (2011). Soon before maize harvesting (June 2018), one sampling point was positioned within the inter-row in the center of each plot (*n* = 16). In NV pseudo-plots, sampling points were positioned in representative locations avoiding the surrounding ant/termite nests and root trees (*n* = 4). The soil water content was measured in all sampling points previously VESS measurements to guarantee similar soil moisture in the treatments and averaged 0.25 g g^{-1} ± 0.004. A small trench sized in ~0.03 m^3 (0.2 m wide x 0.5 m long x 0.3 m deep) was dug out following thorough collection of an undisturbed sample (monolith) sized as ~4,000 cm^3 (0.1 m wide x 0.2 m long x 0.2 m deep) using a flat spade. The monolith was transferred to a tight plastic container enabling the evaluation while preserving its structure. Briefly, the evaluation included in that sequence: (i) removal of residues and clods; (ii) layers identification of contrasting structure; (ii) thickness measurement of identified layers; (iv) gentle hand break up of monolith structure along its fracture lines; (v) fragmentation of some aggregates down to ~2.0 cm to confirm scores; (vi) aggregates morphology scanning; (vii) score layers according to their primary structural quality by comparing its overall structure and that described in the VESS chart (Guimarães et al., 2011); and (viii) take pictures. The score ranges from 1 to 5, in which lower scores (1 and 2) meaning greater structural quality by representing highly porous and rooted with crumbling aggregates, and

higher scores (4 and 5) meaning declined structural quality by representing restricted porosity and no roots with hard clods. Furthermore, we considered score 3 as a threshold from which soil begins to decline its structural quality, meaning that decisions-making regards soil management must be taken (Cherubin et al., 2017).

The scores of each individual layer were integrated into an overall VESS score (Sq) using the Eq. 1.

$$VESS\ Sq = \sum_{i=1}^{n} \frac{Sq_i \times Lt_i}{Mt} \quad \text{Eq. 1}$$

where, Sq_i is the structural quality score of the layer *i*; Lt_i is the thickness of the layer *i* (m), and; *Mt* is the monolith thickness (0.2 m).

In addition, since soil layer with contrasting scores presented different thicknesses in each plot, we normalize their scores through weighted average by setting three layers of standardized thicknesses: topsoil (0-0.1 m), subsurface (0.1-0.2 m) and plough layers (0-0.2 m).

Following the VESS measurements, a soil sampling was performed at each plot at the 0-0.1 and 0.1-0.2 m layers using a manual drilling probe. In addition, undisturbed soil samples were taken beside VESS sampling points using stainless steel cores (~98 cm^3; n = 1 per layer per plot) to evaluate soil physical parameters.

2.2.3 Laboratory soil physical analyses

In the laboratory, the undisturbed samples were used to evaluate parameters (below described) that are typically measured to investigating soil structural changes induced by land use and soil management (Bünemann et al., 2018; Cherubin et al., 2016a; Rabot et al., 2018). All physical analyses were performed following methodologies described by Teixeira et al. (2017). Soil bulk density (BD) was calculated by the ratio of oven-dried soil mass (105 ºC for 24 h) and core inner volume. Soil macroporosity (MaP) was obtained by mass difference of water saturated soil (0 kPa) and -6 kPa-soil water potential. Soil microporosity (MiP) was obtained by mass difference of -6 kPa-soil water potential and oven-dried soil. Soil total porosity (TP) was calculated by the sum of MaP and MiP. Soil water storage capacity (SWSC) was obtained by the volume ratio of -10 kPa-soil water potential and core inner. Soil

aeration capacity (SAC) was obtained by difference between a unit and SWSC, so that SWSC plus SAC equals 1 (Reynolds et al., 2009). A overall proper balance between water storage and air into the soil porosity was fixed as 2/3 and 1/3 respectively for SWSC and SAC (Olness et al., 1998). Soil hydraulic conductivity (HC) was evaluated using a permeameter while maintaining 2.0 cm water column with 10 min-measurements up to steady-state. Soil penetration resistance (PR) was analysed in -6 kPa-soil water potential by a compression bench machine of conical tip (*CT3TM Texture Analyser, Brookfield Amatek*); the mean compression force applied in each sample was calculated by *TexturePro CT* software and then rated by the penetration surface (0.1164 cm^2).

2.2.4 Statistical analyses

Treatments were grouped into agrosystems SF, SN, SM and CR for multiple comparison between them (model 1). Since NV was a reference area assessed as pseudo-replication it was split from treatments grouping and so a complementary model was formed for the purpose of mean comparison between NV against each of the other treatments (model 2). The data are presented as mean value followed by standard error of the mean (SEM). The data residues of both models were tested for normality distribution and homoscedasticity using Shapiro-Wilk's test and Bartlett's test ($p > 0.100$), respectively. The data from VESS measurements and laboratory soil physics analyses were insert in a one–way ANOVA, where *systems* and *blocks* were respectively considered as fixed factors within the statistical models. When the *F*–test showed significances, Tukey's HSD test and Dunnett's test ($p < 0.100$) were applied to identify the differences between the means of the treatments to model 1 and 2, respectively (Suppl. Table 1). A MANOVA was assessed through the principal component analysis (PCA) using laboratory soil physics parameters, including BD, PR, MaP, MiP, HC and SWSC, and VESS Sq. The relative importance of laboratory soil physics parameters over VESS Sq was proceeded through R^2 decomposition by averaging orders at *lmg* method from *relaimpo* package in R. TP and SAC parameters were excluded from PCA and relative importance analyses due to the high collinearity with MaP and MiP ($r > 0.999$) and the congruent results with SWSC, respectively. All statistical analyses and graphs were performed in R (R Core Team, 2019).

2.3 Results

2.3.1 VESS assessment

The VESS measurements detected two distinct layers (1 and 2) under the agrosystems, except at SF in which a third layer was reached in the bottom (Fig. 3 left). In general, all treatments showed a thinner and of higher structural quality in layer 1 (i.e., low VESS Sq) than layer 2. Native vegetation and SM presented a thicker layer 1 of about 0.068 m with lower VESS Sq (1.1 and 2.1) while SN had a very thin layer about 0.032 m of increased VESS Sq (2.75). At layer 2, SF and SN had a contrasting thickness of 0.11 and 0.17 m respectively while the VESS Sq decreased over the CA gradient from 3.8 at SF to 1.9 at NV. The VESS evaluation also detected a declining of structural quality in layer 2 of agrosystems at lower CA such as SF and SN that showed a higher score related to the established threshold (VESS Sq > 3) from which the soil declines its structural quality (Fig. 3 left).

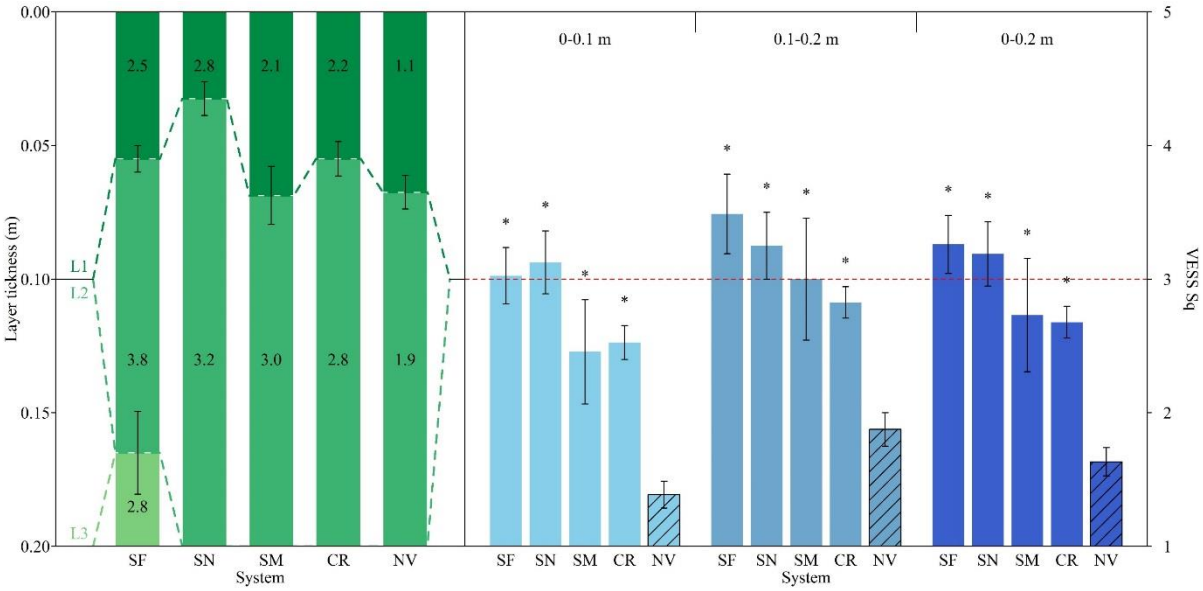


Fig. 3. Thickness and VESS Sq (into the bars) of individual soil layer in the systems (left) and normalized VESS Sq for the 0-0.1, 0.1-0.2 and 0-0.2 m soil layers for each system (right). SF: soybean/fallow under conventional tillage; SN: soybean/fallow under no-till; SM: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; NV: native vegetation. Columns and error bars represent the mean ± SEM. Green-dashed lines (left) indicate the thresholds between underlying layers, where L1, L2 and L3 are the first, second and third layers, respectively. * (right) indicates difference between the respective agrosystem and NV by Dunnett's test ($p < 0.100$). Red line (right) marks the structure quality threshold at VESS Sq = 3

The VESS Sq from individual layers were normalized in three layers of standardized thicknesses at 0-0.1, 0.1-0.2 and 0-0.2 m, in which the results showed effect of agricultural use compared to NV (Fig. 3 right). Although no significant changes among cropping systems were observed, VESS scores were numerically higher in SF and SN (less conservative treatments) compared to SM and CR (more conservative treatments). The NV scores were up to 5.5 and 2.8 times lower than agrosystems in topsoil and subsurface, respectively. In general, even after standardization the VESS Sq showed that the topsoil layer (0-0.10 m) kept a better structural quality than the subsurface layer (0.1-0.2 m). Further, the VESS Sq > 3 found only in layer 2 of SF and SN was dispersed into the standardized layers whereas remained exceeded from the established threshold in topsoil and subsurface.

Differences on soil structure in the treatments caused by soil use, tillage and crop diversity were recorded in images (Fig. 4).

Fig. 4. Images of the soil monolith into a container of the soil structural changes detected by VESS method due to effects of soil use, tillage, and crop diversity. SF: soybean/fallow under conventional tillage; SN: soybean/fallow under no-till; SM: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; NV: native vegetation

2.3.2 Traditional soil physical parameters

Overall, the land transition from Cerrado (NV) to agricultural cropping systems negatively affected the soil physical parameters, regardless of management system and soil layer. On the other hand, changes among different cropping systems were, surprisingly, detected only in subsurface soil layer.

Soil bulk density (BD) had remarkably similar values between layers of agrosystems ranging from 1.19 to 1.31 g cm^{-3} in topsoil and from 1.16 to 1.27 g cm^{-3} in subsurface. The BD at NV was also consistently lower than agrosystems, averaging 1.02 g cm^{-3} in both layers (Fig. 5 left). Although strongly correlated with BD (r = 0.846;

p < 0.001), soil penetration resistance (PR) presented a decreasing of 26, 36, 32 and 46% from topsoil to subsurface at SF, SN, SM and NV while CR remained about 2.16 MPa in both layers. NV had the lowest PR in both topsoil (1.31 MPa) and subsurface (0.90 MPa) layers, different from SM and CR. The PR results suggest rather grouping into agrosystems of minimum and improved CA techniques at decreased and increased PR in both layers as well as in VESS Sq results (Fig. 5 right).

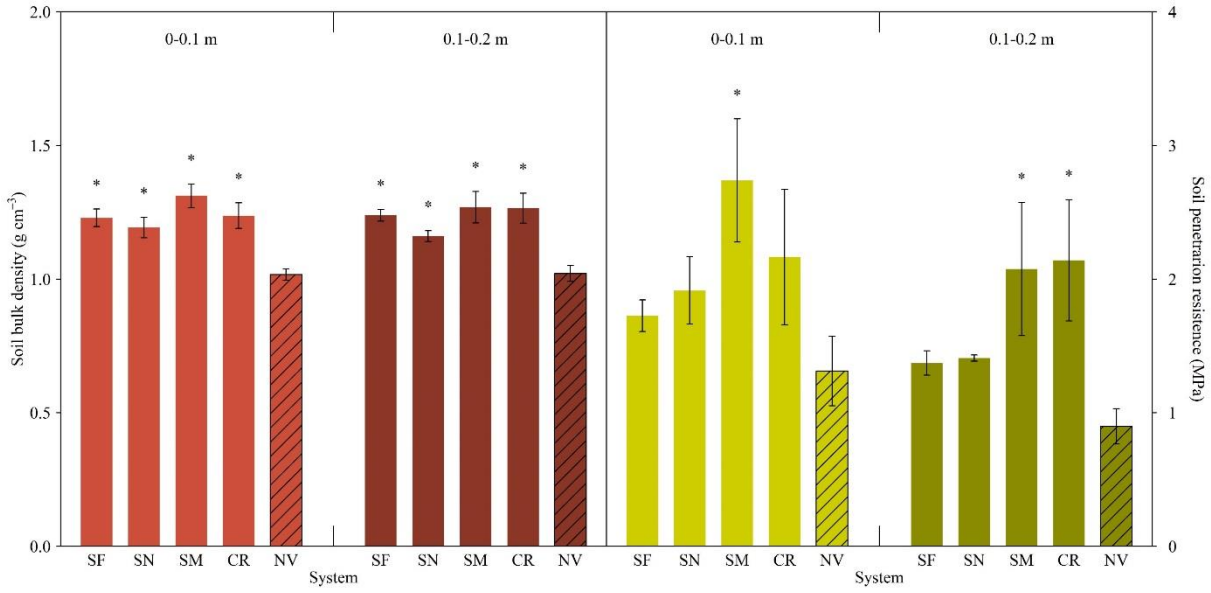


Fig. 5. Soil bulk density (BD, left) and penetration resistance (PR, right) of agroecosystems at 0-0.1 and 0.1-0.2 m layers. SF: soybean/fallow under conventional tillage; SN: soybean/fallow under no-till; SM: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; NV: native vegetation. Columns and error bars represent the mean ± SEM. * indicates difference between the respective agrosystem and NV by Dunnett's test (*p* < 0.100)

The macro- and microporosity (MaP and MiP) were negatively correlated (r = -0.790; *p* < 0.001). MiP had low variation into and between layers, ranging from 0.40 to 0.43 cm^3 cm^{-3} in topsoil and from 0.39 to 0.43 cm^3 cm^{-3} in subsurface and showing no differences among treatments, even comparing agrosystems with NV (Fig. 6 left). However, the MaP in topsoil was greater at NV (0.19 cm^3 cm^{-3}) than SM (0.08 cm^3 cm^{-3}) and CR (0.11 cm^3 cm^{-3}). In subsurface, NV and SN (0.21 and 0.18 cm^3 cm^{-3}) had increased MaP compared to SM and CR (0.10 cm^3 cm^{-3}) (Fig. 6 left). Since MaP and soil water storage capacity (SWSC) were obtained by mass difference at -6 kPa- and -10 kPa-soil water potential, they were strongly correlated (r = -0.972; *p* < 0.001) and so with analogous results (Fig. 6 right). The SWSC at NV were lower than SF, SM and

CR and below the established threshold of 0.66 cm^3 cm^{-3} in topsoil (0.57 cm^3 cm^{-3}) and subsurface (0.52 cm^3 cm^{-3}). That is because NV had higher total porosity while holding similar MiP as in the agrosystems. In topsoil, the SWSC of agrosystems remained above 0.66 cm^3 cm^{-3} while in subsurface only the SN was below that threshold and lower than SM. Soil aeration capacity (SAC) results were supplementary to SWSC ones, since this parameter is associated with SWSC, but in inverse order (Fig. 6 right).

The hydraulic conductivity (HC) showed clear differences among agrosystems and NV in both layers. Surprisingly, the HC in NV was five-fold higher than agrosystems (Fig. 7). Unlike at CR, all treatments increased the HC from topsoil to subsurface, with lower increments at SN (3.2-4.1 cm h^{-1}) and higher at NV (15.8-19.2 cm h^{-1}). Comparing subsurface layer of the agrosystems, SN had superior HC than CR by about six times.

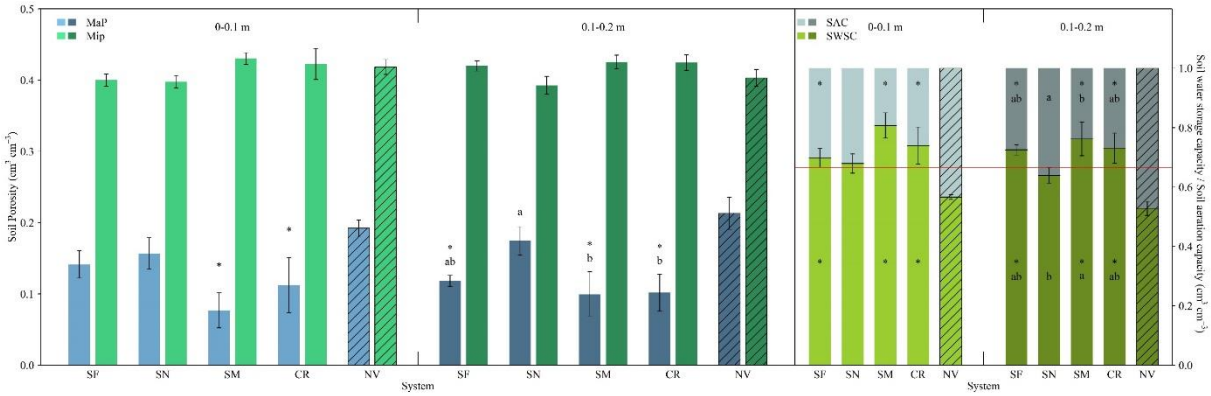


Fig. 6. Soil macro- and microporosity (MaP and MiP, left) and soil water storage and air capacity (SWSC and SAC, right) of agroecosystems at 0-0.1 and 0.1-0.2 m layers. SF: soybean/fallow under conventional tillage; SN: soybean/fallow under no-till; SM: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; NV: native vegetation. Columns and error bars represent the mean ± SEM. * indicates difference between the respective agrosystem and NV according to Dunnett's test (p < 0.100). Lowercase letters into each column indicate differences among agrosystems according to Tukey's test (p < 0.100). Red-solid line indicates a threshold relation for proper balance between water storage and air into the soil porous space (SWSC:SAC = 0.66:0.33 cm^3 cm^{-3})

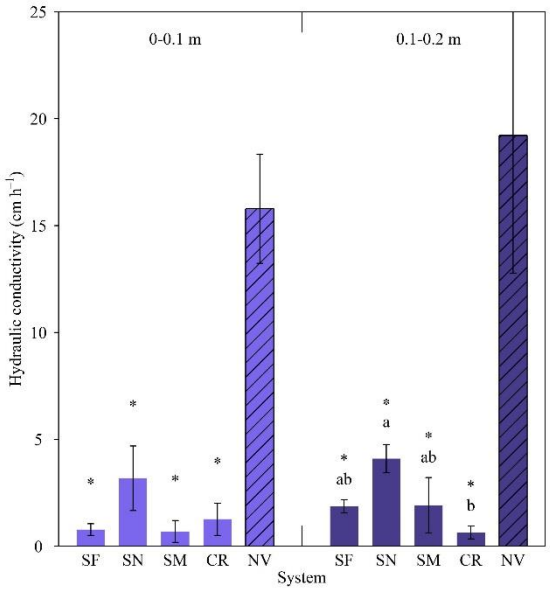


Fig. 7. Hydraulic conductivity of soil (HC) of agroecosystems at 0-0.1 and 0.1-0.2 m layers. SF: soybean/fallow under conventional tillage; SN: soybean/fallow under no-till; SM: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; NV: native vegetation. Columns and error bars represent the mean ± SEM. * indicates difference between the respective agrosystem and NV according to Dunnett's test ($p < 0.100$). Lowercase letters into each column indicate differences among agrosystems according to Tukey's test ($p < 0.100$)

2.3.3 PCA and relative importance

A multivariate principal component analysis (PCA) revealed that the first two principal components (PC1+PC2) accounted for 89.1% of the variation of soil physical parameters and only the PC1 explained 69.5% of such variation (Fig. 8). SWSC, BD and MaP were the major loadings at PC1 axis while VESS Sq and MiP were the major loadings at PC2 axis. Considering PC1 and PC2, positive correlations were detected among SWSC, PR and MiP (quadrant I) and among VESS and BD (quadrant IV) that in turn were negatively correlated to MaP (quadrant III) and HC (quadrant II), respectively. The loadings directions and magnitudes also affected the distribution of the mean groups scores showing that HC was the variable responsible for the distinguishing of NV from agrosystems. Likewise, MaP and VESS pushed SF and SN away from SM and CR.

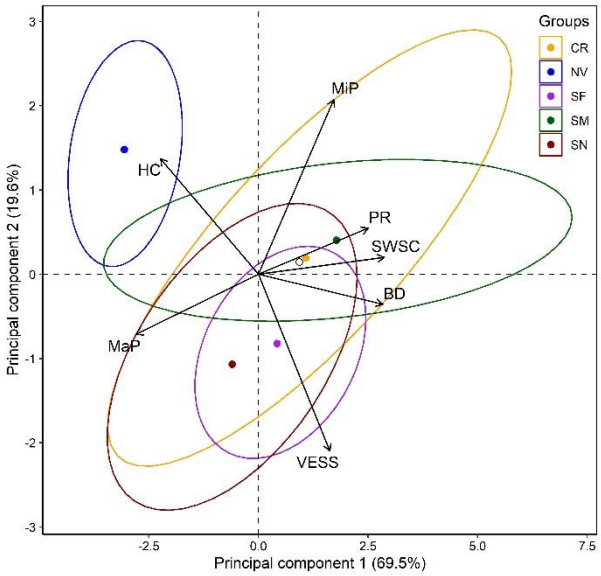


Fig. 8. Principal component analysis (PCA) of agroecosystems based on soil physical attributes at 0-0.1 and 0.1-0.2 m. SF: soybean/fallow under conventional tillage; SN: soybean/fallow under no-till; SM: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; NV: native vegetation. BD: soil bulk density; PR: soil penetration resistance; MaP soil macroporosity; MiP soil microporosity; SWSC: soil water storage capacity; HC: hydraulic conductivity of soil; VESS: visual evaluation of soil structure. Percentages in the axis labels indicate the amount of variance explained by each principal component. Coloured circles represent mean values of scores groups derived from the results of individual sample (n = 20 + 20) and the respective concentration ellipses assume a multivariate *t*-distribution (n = 8). Arrows indicate the loadings of variables

To test the relative importance of the soil physical properties as predictors of the VESS Sq, a linear model revealed that BD (30.5%) was the mainly variable driving the VESS Sq, followed by MaP (18.1%) and SWSC (16.0%); the three variables together accounted for 64.6% of capacity of prediction (Fig. 9). Unexpectedly, PR values showed a limited potential (7.1%) as predictor of the VESS Sq.

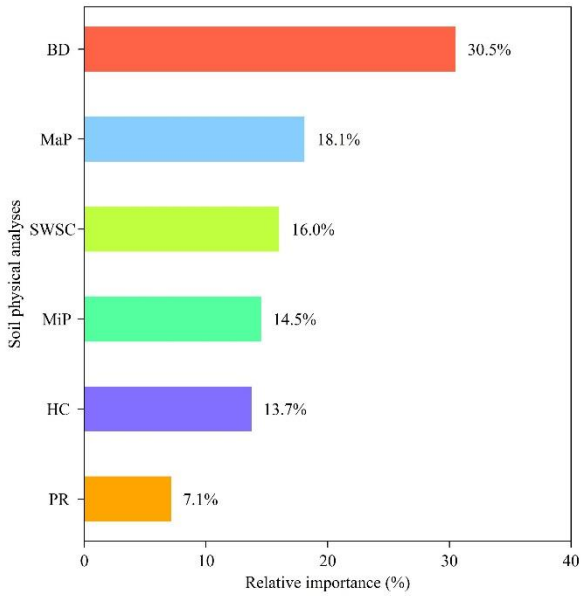


Fig. 9. Relative importance (%) of soil physical attributes over VESS Sq based on a linear model of R^2 decomposition. BD: soil bulk density; PR: soil penetration resistance; MaP soil macroporosity; MiP soil microporosity; SWSC: soil water storage capacity; HC: hydraulic conductivity of soil

2.4 Discussion

2.4.1 Effect of agricultural use on physical soil quality

Both visual and laboratory methods clearly showed that agricultural use declined physical soil quality compared to soil under NV (Fig. 5, 6 and 7). This is in accordance with previous evidences showing that land use change (LUC) from native vegetation to agrosystems increases bulk density and soil penetration resistance while decrease the macroporosity and hydraulic conductivity of the soil (Carneiro et al., 2009; Cherubin et al., 2016b; Gomes et al., 2016; Hunke et al., 2015; Rojas et al., 2016). In a review of 80 studies performed from 1977 to 2012, Hunke et al. (2015) found that croplands had significantly higher soil bulk density than the native Cerrado sites. The LUC towards an modern agricultural system based on production of commodities with high investments in technologies and inputs for achieving high yields is unequivocally linked to soil compaction due to the intensification of the machinery traffic (Batey, 2009; Keller et al., 2019; Smith et al., 2016). Furthermore, 23-90% of the potentially problematic traffic operations incur a high risk of subsoil compaction, particularly the harvesting operations (Thorsøe et al., 2019). Although evidenced, the reduction in crop diversity found in intensified agrosystems can also be responsible for the degradation of physical soil quality (Gould et al., 2016).

The NV distancing from the treatments on agrosystems in PCA confirm the LUC effect mainly by the loading of the HC and MaP parameters at PC1 (Fig. 8). By definition, HC is mainly influenced by the shape and continuity of the porous space, specially macropores, which in turn is preferentially affected by compaction and can undergo changes in the orientation of the porous system from vertical to horizontal flow (Gregory et al., 2015). Such dependency of MaP by HC strengthens the effect of LUC from NV to agrosystems.

The VESS method also detected a reduction in physical soil quality in the treatments under agrosystems in both layers (Fig. 3). It agrees with a recent meta-analysis conducted by Franco et al. (2019) who showed that native vegetation soils presented lower VESS scores (greater soil structural quality) than agricultural soils. These VESS results are confirmed by traditional physical indicators (Cavalcanti et al., 2020; Cherubin et al., 2017). Comparison between native vegetation soils and agrosystems soils is an important strategy to stablish a reference of soil quality and detect if and how much the management system is degrading it (Ball et al., 2017).

2.4.2 Effect of management practices on physical soil quality

The soil physical parameters MaP, SWSC, SAC and HC were impacted by management practices, however, those changes occurred only in subsurface layer. On average, all soil physical parameters from subsoil of agrosystems were slightly improved comparing to topsoil. That was not surprisingly since others studies also have found similar results on soil physical parameters in which they attribute them to the more pronounced effects of machinery over the topsoil (Blanco-Canqui et al., 2010; Castioni et al., 2018; de Sant-Anna et al., 2017; Jantalia et al., 2007; Sisti et al., 2004). More importantly, adoption of crop diversification (SN, SM and CR) impacted more significantly the soil physical parameters than the modification in the tillage system since SF and SN presented similar results (Fig. 5 and 6).

The effect of conventional tillage and no-tillage on soil physical parameters was recently revised by Blanco-Canqui and Ruis (2018). The authors found that, in most cases, there is no improvements in soil physical parameters such as soil bulk density, penetration resistance and hydraulic conductivity by the adoption of no-tillage. However, soil bulk density, penetration resistance and hydraulic conductivity were improved by no- tillage in 19, 9, and 33% of the studies, respectively. Likewise, Franco et al. (2019) in a global meta-analysis of studies using the VESS method, observed

that soil tillage management did not change VESS scores (i.e., no-tillage and conventional tillage scores ranged from 2.2-2.5 and 2.4-2.7, respectively). Even though there were no differences between VESS Sq of SF and SN in our study, we detected a declining on soil structure quality (VESS Sq > 3) at both layers due to monocropping adoption.

Overall, tillage management may positively affect soil physical parameters with the extent depending on soil textural class and time of no-tillage adoption (Blanco-Canqui and Ruis, 2018). On our study, +9 years of management under a ~70%-clay soil was not enough to print great differences between tillage management.

The increasing of crop diversity under no-tillage from monoculture (SN) to crop rotation with four crops in rotation (CR) and passing by double cropping (SM) showed a mixed effect on the soil physical parameters (Fig. 5, 6 and 7). Our results contrast with the findings of Blanco-Canqui et al. (2015) whose observed that adding cover crops reduced soil penetration resistance and bulk density in most cases of temperate soils. However, these benefits were no longer observed by Munkholm et al. (2013) and Moraes et al. (2016), when evaluating the effects of crop succession and rotation on soil quality after decades of adoption. Nevertheless, Munkholm et al. (2013) found a positive effect of crop rotation on VESS scores, as found in our study. The difference between the results obtained in current study and those found in the literature can be explained by the fact that clayey soils are more prone to compaction caused by machinery traffic (de Lima et al., 2017) and the increased frequency of machinery traffic intensify the compaction processes (Hamza and Anderson, 2005; Keller et al., 2019). Although we have hypothesized that no-tillage and crop rotation would be suitable strategies to improve soil structure and mitigate soil physical degradation, it appears that the machinery traffic masked the potential benefits of crop diversification on soil physical parameters. That support the declined findings mostly on SM and CR in which 35 and 29 total operations were run, respectively. In addition, clayey soils also can be more resilient to compaction, especially oxidic Oxisol, partially recovering the values of soil strength , bulk density and macroporosity after wetting-drying cycles (de Andrade Bonetti et al., 2017; Gregory et al., 2007). That may support the superior performance of SN among the agrosystems on MaP, SWSC, SAC and HC added to the lower machinery operations frequency (total = 18) in which a seven-months fallow per season showed more beneficial to soil physical quality even compared to annual heavy-disc harrowing. Despite the negative effects of including a more diverse crop

sequence in the physical soil parameters (Fig. 6 and 7), there were advantages such as improvements in land profitability and in soil biological parameters that are out of the scope of the current study but must be considered by the end-user (not published yet).

Detecting soil changes in diversified cropping systems in not a simple task, because tradeoffs and synergies associated with growing and management multiple crops occur simultaneously in a complex way, and also are time depend (McDaniel et al., 2014; Reich et al., 2012b; Zegada-Lizarazu and Monti, 2011). On our study, after +9 years experiencing crop diversification was not enough to detect positive effects on physical soil quality and neither on chemical at all (not published yet). It shows the relevance to conduct long-term experiments to design more productive, profitable, and sustainable cropping system. We also emphasized that potential detrimental impacts on soil physical quality on intensified agrosystems can be mitigated through complementary practices such as controlled traffic farming in no-till areas (Blanco-Canqui et al., 2010; Hamza and Anderson, 2005).

2.4.3 Sensibility of VESS scores for monitoring physical quality in Cerrado agricultural systems

The VESS scores detected a surface layer with better soil physical condition to plant growth. It has been associated with greater soil aggregation induced by C inputs via crop residues (Blanco-Canqui et al., 2015), higher presence of biopores formed by soil fauna activity and decomposition of roots (Kuzyakov and Blagodatskaya, 2015) and also, mechanical action of seeder furrow that alleviates soil compaction in the first centimeters (Ferreira et al., 2020; Moreira et al., 2020). The sensibility of VESS method has been recognized to detect soil physical quality under a spread influence of climate, soil texture, tillage and crop diversity (Franco et al., 2019). On this study, the VESS Sq proved to be effective to quantify the soil structure quality under different LUC and recognizing the agrosystems with potential to decline physical soil quality mainly due to subsurface compaction. However, the visual method did not detect changes induced by crop diversity, that warrant just part of the results from physical parameters (Fig. 3). In our understanding, VESS method is complementary to traditional soil physical analyses and both analyses are important for a better evaluation of soil physical changes in different scales and levels of details. Ultimately, to some extent, the relative importance of VESS Sq contributes to our previous statement because it showed that

VESS Sq covered without great discrepancies a series of quantitative information (six laboratory parameters) in a semi-qualitative assessment for the soil structural quality and could not be represented by just a single one. In this way, VESS has been considered an integrative method that allows to have a first evaluation of soil physical quality in quick, cheap and simple manner (Castioni et al., 2018; Cherubin et al., 2017). It can be useful for farmers and consultants to monitor and identify early stages of soil physical degradation, and consequently support decision of changes in the management practices.

Studies comparing VESS scores with laboratory parameters highlighted that undisturbed samples do not necessarily faithfully match over the respective layers identified by the visual method (Cherubin et al., 2017, 2016b; Guimarães et al., 2017, 2013). It happened due to the core stratification for undisturbed sampling to a certain depth which assumed that the sampled core represented the larger layer (e.g., a 5-cm diameter core sampled at 0.1 m depth to represent a 0-0.2 m layer). Thus, it is common for the core stratification to be partially or even totally mismatched from the layer identified by VESS. At this point, the visual method had an advantage because it identified exactly the layers considering the soil structure in the evaluated profile instead of stratifying layers that would result in biased measurements. Therefore, we encourage that future studies use previously the VESS method to identify soil layers with distinct structural quality and then, collect undisturbed soil samples for laboratory physical tests in that same layers visually identified. Certainly, it is a good example of complementarity of on-farm visual evaluations and quantitative laboratorial measurements.

2.5 Conclusions

Compared to soils under native vegetation, intensively cropped soils present degradation of soil physical quality that was not recovered after +9 years of conservation management adoption such as no-till and crop diversification. From the soil physical parameters evaluated, the hydraulic conductivity was the most affected by conversion of native vegetation to agrosystems.

The limited time of different management adopted, linked to the high resilience of the ~70% clay content Oxisol, may explain the non-significant changes in most of the physical soil parameters between soil tillage (conventional versus no-tillage) and crop diversity observed herein. The slight decline in soil physical quality promoted by the

multi-cropping compared to monoculture can be related to the higher intensity of machinery operations required to manage a more diverse crop sequence.

The VESS approach successfully identified changes in the soil structure induced by the soil use whereas laboratory analysis detected changes in specific functions, such as related to the soil water retention and hydraulic conductivity. In fact, we consider VESS as a powerful assessment for farmers due to its quick, cheap, and direct diagnosis for management purposes while laboratory tests as fundamental to better understand changes in specific soil processes and functions. Ultimately, the different approaches are synergistic and complementary to monitor the soil physical quality in different agroecosystems.

References

Alvares, C.A., Stape, J.L., Sentelhas, P.C., de Moraes Gonçalves, J.L., 2013. Modeling monthly mean air temperature for Brazil. Theor. Appl. Climatol. 113, 407–427. https://doi.org/10.1007/s00704-012-0796-6

Anghinoni, G., Tormena, C.A., Lal, R., Zancanaro, L., Kappes, C., 2019. Enhancing soil physical quality and cotton yields through diversification of agricultural practices in central Brazil. L. Degrad. Dev. 1–11. https://doi.org/10.1002/ldr.3267

Ball, B.C., Batey, T., Munkholm, L.J., 2007. Field assessment of soil structural quality - a development of the Peerdklamp test. Soil Use Manag. 23, 329–337. https://doi.org/10.1111/j.1473-2743.2007.00102.x

Ball, B.C., Guimarães, R.M.L., Cloy, J.M., Hargreaves, P.R., Shepherd, T.G., McKenzie, B.M., 2017. Visual soil evaluation: A summary of some applications and potential developments for agriculture. Soil Tillage Res. 173, 114–124. https://doi.org/10.1016/j.still.2016.07.006

Batey, T., 2009. Soil compaction and soil management - A review. Soil Use Manag. 25, 335–345. https://doi.org/10.1111/j.1475-2743.2009.00236.x

Blanco-Canqui, H., Claassen, M.M., Stone, L.R., 2010. Controlled Traffic Impacts on Physical and Hydraulic Properties in an Intensively Cropped No-Till Soil. Soil Sci. Soc. Am. J. 74, 2142–2150. https://doi.org/10.2136/sssaj2010.0061

Blanco-Canqui, H., Ruis, S.J., 2018. No-tillage and soil physical environment. Geoderma 326, 164–200. https://doi.org/10.1016/j.geoderma.2018.03.011

Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A., Hergert, G.W., 2015. Cover crops and ecosystem services: Insights from studies in temperate soils. Agron. J. 107, 2449–2474. https://doi.org/10.2134/agronj15.0086

Bronick, C.J., Lal, R., 2005. Soil structure and management: A review. Geoderma 124, 3–22. https://doi.org/10.1016/j.geoderma.2004.03.005

Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., De Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J.W., Brussaard, L., 2018. Soil quality – A critical review. Soil Biol. Biochem. 120, 105–125. https://doi.org/10.1016/j.soilbio.2018.01.030

Carneiro, M.A.C., Souza, E.D., Reis, E.F., Pereira, H.S., Azevedo, W.R., 2009. Atributos físicos, químicos e biológicos de solo de cerrado sob diferentes sistemas de uso e manejo. Rev. Bras. Cienc. do Solo 33, 147–157.

Castioni, G.A., Cherubin, M.R., Menandro, L.M.S., Sanches, G.M., Bordonal, R. de O., Barbosa, L.C., Franco, H.C.J., Carvalho, J.L.N., 2018. Soil physical quality response to sugarcane straw removal in Brazil: A multi-approach assessment. Soil Tillage Res. 184, 301–309. https://doi.org/10.1016/j.still.2018.08.007

Cavalcanti, R.Q., Rolim, M.M., de Lima, R.P., Tavares, U.E., Pedrosa, E.M.R., Cherubin, M.R., 2020. Soil physical changes induced by sugarcane cultivation in the Atlantic Forest biome, northeastern Brazil. Geoderma 370, 114353. https://doi.org/10.1016/j.geoderma.2020.114353

Cavalieri, K.M.V., da Silva, A.P., Tormena, C.A., Leão, T.P., Dexter, A.R., Håkansson, I., 2009. Long-term effects of no-tillage on dynamic soil physical properties in a Rhodic Ferrasol in Paraná, Brazil. Soil Tillage Res. 103, 158–164. https://doi.org/10.1016/j.still.2008.10.014

Cherubin, M.R., Franco, A.L.C., Guimarães, R.M.L., Tormena, C.A., Cerri, C.E.P., Karlen, D.L., Cerri, C.C., 2017. Assessing soil structural quality under Brazilian sugarcane expansion areas using Visual Evaluation of Soil Structure (VESS). Soil Tillage Res. 173, 64–74. https://doi.org/10.1016/j.still.2016.05.004

Cherubin, M.R., Karlen, D.L., Franco, A.L.C., Cerri, C.E.P., Tormena, C.A., Cerri, C.C., 2016a. A soil management assessment framework (SMAF) evaluation of brazilian sugarcane expansion on soil quality. Soil Sci. Soc. Am. J. 80, 215–226. https://doi.org/10.2136/sssaj2015.09.0328

Cherubin, M.R., Karlen, D.L., Franco, A.L.C., Tormena, C.A., Cerri, C.E.P., Davies, C.A., Cerri, C.C., 2016b. Soil physical quality response to sugarcane expansion in Brazil. Geoderma 267, 156–168. https://doi.org/10.1016/j.geoderma.2016.01.004

Coelho, M.J.A., 2019. Legacy phosphorus accumulation under long-term soil and fertilizer management in crop production Piracicaba 2019.

de Andrade Bonetti, J., Anghinoni, I., de Moraes, M.T., Fink, J.R., 2017. Resilience of soils with different texture, mineralogy and organic matter under long-term conservation systems. Soil Tillage Res. 174, 104–112. https://doi.org/10.1016/j.still.2017.06.008

de Lima, R.P., da Silva, A.P., Giarola, N.F.B., da Silva, A.R., Rolim, M.M., 2017. Changes in soil compaction indicators in response to agricultural field traffic. Biosyst. Eng. 162, 1–10. https://doi.org/10.1016/j.biosystemseng.2017.07.002

de Sant-Anna, S.A.C., Jantalia, C.P., Sá, J.M., Vilela, L., Marchão, R.L., Alves, B.J.R., Urquiaga, S., Boddey, R.M., 2017. Changes in soil organic carbon during 22 years of pastures, cropping or integrated crop/livestock systems in the Brazilian Cerrado. Nutr. Cycl. Agroecosystems 108, 101–120. https://doi.org/10.1007/s10705-016-9812-z

Emmet-Booth, J.P., Forristal, P.D., Fenton, O., Ball, B.C., Holden, N.M., 2016. A review of visual soil evaluation techniques for soil structure. Soil Use Manag. 32, 623–634. https://doi.org/10.1111/sum.12300

Ferreira, A. de O., Sá, J.C. de M., Lal, R., Tivet, F., Briedis, C., Inagaki, T.M., Gonçalves, D.R.P., Romaniw, J., 2018. Macroaggregation and soil organic carbon restoration in a highly weathered Brazilian Oxisol after two decades under no-till. Sci. Total Environ. 621, 1559–1567. https://doi.org/10.1016/j.scitotenv.2017.10.072

Ferreira, C.J.B., Tormena, C.A., Severiano, E.D.C., Zotarelli, L., Betioli Júnior, E., 2020. Soil compaction influences soil physical quality and soybean yield under long-term no-tillage. Arch. Agron. Soil Sci. 00, 1–14. https://doi.org/10.1080/03650340.2020.1733535

Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O'Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockström, J., Sheehan, J., Siebert, S., Tilman, D., Zaks, D.P.M., 2011. Solutions for a cultivated planet. Nature 478, 337–342. https://doi.org/10.1038/nature10452

Franco, H.H.S., Guimarães, R.M.L., Tormena, C.A., Cherubin, M.R., Favilla, H.S., 2019. Global applications of the Visual Evaluation of Soil Structure method: A systematic review and meta-analysis. Soil Tillage Res. 190, 61–69. https://doi.org/10.1016/j.still.2019.01.002

Gomes, J.B.V., Fernandes, M.F., Pacheco, E.P., Barreto, A.C., Norton, L.D., Curi, N., 2016. Calcareous soils in the Northeastern of Brazil: Alterations of attributes from different land use history. Soil Tillage Res. 155, 27–34. https://doi.org/10.1016/j.still.2015.07.010

Gould, I.J., Quinton, J.N., Weigelt, A., De Deyn, G.B., Bardgett, R.D., 2016. Plant diversity and root traits benefit physical properties key to soil function in grasslands. Ecol. Lett. 19, 1140–1149. https://doi.org/10.1111/ele.12652

Gregory, A.S., Ritz, K., Mcgrath, S.P., Quinton, J.N., Goulding, K.W.T., Jones, R.J.A., Harris, J.A., Bol, R., Wallace, P., Pilgrim, E.S., Whitmore, A.P., 2015. A review of the impacts of degradation threats on soil properties in the UK. Soil Use Manag. 31, 1–15. https://doi.org/10.1111/sum.12212

Gregory, A.S., Watts, C.W., Whalley, W.R., Kuan, H.L., Griffiths, B.S., Hallett, P.D., Whitmore, A.P., 2007. Physical resilience of soil to field compaction and the interactions with plant growth and microbial community structure. Eur. J. Soil Sci. 58, 1221–1232. https://doi.org/10.1111/j.1365-2389.2007.00956.x

Guedes Filho, O., da Silva, A.P., Giarola, N.F.B., Tormena, C.A., 2013. Structural properties of the soil seedbed submitted to mechanical and biological chiseling under

no-tillage. Geoderma 204–205, 94–101. https://doi.org/10.1016/j.geoderma.2013.04.017

Guimarães, R.M.L., Ball, B.C., Tormena, C.A., 2011. Improvements in the visual evaluation of soil structure. Soil Use Manag. 27, 395–403. https://doi.org/10.1111/j.1475-2743.2011.00354.x

Guimarães, R.M.L., Ball, B.C., Tormena, C.A., Giarola, N.F.B., da Silva, álvaro P., 2013. Relating visual evaluation of soil structure to other physical properties in soils of contrasting texture and management. Soil Tillage Res. 127, 92–99. https://doi.org/10.1016/j.still.2012.01.020

Guimarães, R.M.L., Neves Junior, A.F., Silva, W.G., Rogers, C.D., Ball, B.C., Montes, C.R., Pereira, B.F.F., 2017. The merits of the Visual Evaluation of Soil Structure method (VESS) for assessing soil physical quality in the remote, undeveloped regions of the Amazon basin. Soil Tillage Res. https://doi.org/10.1016/j.still.2016.10.014

Hamza, M.A., Anderson, W.K., 2005. Soil compaction in cropping systems: A review of the nature, causes and possible solutions. Soil Tillage Res. 82, 121–145. https://doi.org/10.1016/j.still.2004.08.009

Han, E., Kautz, T., Köpke, U., 2016. Precrop root system determines root diameter of subsequent crop. Biol. Fertil. Soils 52, 113–118. https://doi.org/10.1007/s00374-015-1049-5

Han, E., Kautz, T., Perkons, U., Uteau, D., Peth, S., Huang, N., Horn, R., Köpke, U., 2015. Root growth dynamics inside and outside of soil biopores as affected by crop sequence determined with the profile wall method. Biol. Fertil. Soils 51, 847–856. https://doi.org/10.1007/s00374-015-1032-1

Hunke, P., Mueller, E.N., Schröder, B., Zeilhofer, P., 2015. The Brazilian Cerrado: Assessment of water and soil degradation in catchments under intensive agricultural use. Ecohydrology 8, 1154–1180. https://doi.org/10.1002/eco.1573

Jantalia, C.P., Resck, D.V.S., Alves, B.J.R., Zotarelli, L., Urquiaga, S., Boddey, R.M., 2007. Tillage effect on C stocks of a clayey Oxisol under a soybean-based crop rotation in the Brazilian Cerrado region. Soil Tillage Res. 95, 97–109. https://doi.org/10.1016/j.still.2006.11.005

Kassam, A., Friedrich, T., Derpsch, R., 2019. Global spread of Conservation Agriculture. Int. J. Environ. Stud. 76, 29–51. https://doi.org/10.1080/00207233.2018.1494927

Kassam, A., Friedrich, T., Derpsch, R., Kienzle, J., 2015. Overview of the Worldwide Spread of Conservation Agriculture. F. Actions Sci. Reports. 8, 1–11.

Keller, T., Sandin, M., Colombi, T., Horn, R., Or, D., 2019. Historical increase in agricultural machinery weights enhanced soil stress levels and adversely affected soil functioning. Soil Tillage Res. 194, 104293. https://doi.org/10.1016/j.still.2019.104293

Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: Concept & review. Soil Biol. Biochem. 83, 184–199. https://doi.org/10.1016/j.soilbio.2015.01.025

Lal, R., 2015. Sequestering carbon and increasing productivity by conservation agriculture. J. Soil Water Conserv. 70, 55A-62A. https://doi.org/10.2489/jswc.70.3.55a

Landl, M., Schnepf, A., Uteau, D., Peth, S., Athmann, M., Kautz, T., Perkons, U., Vereecken, H., Vanderborght, J., 2019. Modeling the Impact of Biopores on Root Growth and Root Water Uptake. Vadose Zo. J. 18, 0. https://doi.org/10.2136/vzj2018.11.0196

McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? a meta-analysis. Ecol. Appl. 24, 560–570. https://doi.org/10.1890/13-0616.1

Moraes, M.T., Debiasi, H., Carlesso, R., Cezar Franchini, J., Rodrigues da Silva, V., Bonini da Luz, F., 2016. Soil physical quality on tillage and cropping systems after two decades in the subtropical region of Brazil. Soil Tillage Res. 155, 351–362. https://doi.org/10.1016/j.still.2015.07.015

Moreira, W.H., Tormena, C.A., de Lima, R.P., Anghinoni, G., Imhoff, S., 2020. The influence of sowing furrow opening and wetting and drying cycles on soil physical quality under no-tillage in Southern Brazil. Soil Tillage Res. 204. https://doi.org/10.1016/j.still.2020.104711

Munkholm, L.J., Heck, R.J., Deen, B., 2013. Long-term rotation and tillage effects on soil structure and crop yield. Soil Tillage Res. 127, 85–91. https://doi.org/10.1016/j.still.2012.02.007

Olness, A., Clapp, C.E., Liu, R., Palazzo, A.J., 1998. Biosolids and their effects on soil properties, in: Handbook of Soil Conditioners. pp. 141–165.

Pires, C.A.B., Amado, T.J.C., Reimche, G., Schwalbert, R., Sarto, M.V.M., Nicoloso, R.S., Fiorin, J.E., Rice, C.W., 2020. Diversified crop rotation with no-till changes microbial distribution with depth and enhances activity in a subtropical Oxisol. Eur. J. Soil Sci. 1–15. https://doi.org/10.1111/ejss.12981

R Core Team, 2019. R: A language and environment for statistical computing.

Rabot, E., Wiesmeier, M., Schlüter, S., Vogel, H.J., 2018. Soil structure as an indicator of soil functions: A review. Geoderma 314, 122–137. https://doi.org/10.1016/j.geoderma.2017.11.009

Reich, P.B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S.E., Flynn, D.F.B., Eisenhauer, N., 2012a. Impacts of biodiversity loss escalate through time as redundancy fades. Science (80-.). 336, 589–592. https://doi.org/10.1126/science.1217909

Reich, P.B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S.E., Flynn, D.F.B., Eisenhauer, N., 2012b. Impacts of biodiversity loss escalate through time as redundancy fades. Science (80-.). 336, 589–592. https://doi.org/10.1126/science.1217909

Reynolds, W.D., Drury, C.F., Tan, C.S., Fox, C.A., Yang, X.M., 2009. Use of indicators and pore volume-function characteristics to quantify soil physical quality. Geoderma 152, 252–263. https://doi.org/10.1016/j.geoderma.2009.06.009

Rojas, J.M., Prause, J., Sanzano, G.A., Arce, O.E.A., Sánchez, M.C., 2016. Soil quality indicators selection by mixed models and multivariate techniques in deforested areas for agricultural use in NW of Chaco, Argentina. Soil Tillage Res. 155, 250–262. https://doi.org/10.1016/j.still.2015.08.010

Sá, J.C. de M., Lal, R., Cerri, C.C., Lorenz, K., Hungria, M., de Faccio Carvalho, P.C., 2017. Low-carbon agriculture in South America to mitigate global climate change and advance food security. Environ. Int. 98, 102–112. https://doi.org/10.1016/j.envint.2016.10.020

Sisti, C.P.J., Dos Santos, H.P., Kohhann, R., Alves, B.J.R., Urquiaga, S., Boddey, R.M., 2004. Change in carbon and nitrogen stocks in soil under 13 years of conventional or zero tillage in southern Brazil. Soil Tillage Res. 76, 39–58. https://doi.org/10.1016/j.still.2003.08.007

Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res. 79, 7–31. https://doi.org/10.1016/j.still.2004.03.008

Smith, P., House, J.I., Bustamante, M., Sobocká, J., Harper, R., Pan, G., West, P.C., Clark, J.M., Adhya, T., Rumpel, C., Paustian, K., Kuikman, P., Cotrufo, M.F., Elliott, J.A., Mcdowell, R., Griffiths, R.I., Asakawa, S., Bondeau, A., Jain, A.K., Meersmans, J., Pugh, T.A.M., 2016. Global change pressures on soils from land use and management. Glob. Chang. Biol. 22, 1008–1028. https://doi.org/10.1111/gcb.13068

Soil Survey Staff, 2014. Keys to soil taxonomy, Soil Conservation Service.

Teixeira, P.C., Donagemma, G.K., Fontana, A.I., Teixeira, W.G., 2017. Manual de métodos de analise de solo, EMBRAPA. https://doi.org/1517-2627

Thorsøe, M.H., Noe, E.B., Lamandé, M., Frelih-Larsen, A., Kjeldsen, C., Zandersen, M., Schjønning, P., 2019. Sustainable soil management - Farmers' perspectives on subsoil compaction and the opportunities and barriers for intervention. Land use policy 86, 427–437. https://doi.org/10.1016/j.landusepol.2019.05.017

Tuchtenhagen, I.K., Lima, C.L.R. de, Bamberg, A.L., Guimarães, R.M.L., Mansonia, P.-M., 2018. Visual Evaluation of the Soil Structure under Different Management Systems in Lowlands in Southern Brazil. Rev. Bras. Ciência do Solo 42, 1–13. https://doi.org/10.1590/18069657rbcs20170270

van Raij, B., Andrade, J.C., Cantarella, H., Quaggio, J.A., 2001. Análise química para avaliação da fertilidade de solos tropicais.

Venter, Z.S., Jacobs, K., Hawkins, H.J., 2016. The impact of crop rotation on soil microbial diversity: A meta-analysis. Pedobiologia (Jena). 59, 215–223. https://doi.org/10.1016/j.pedobi.2016.04.001

Xue, J.F., Pu, C., Liu, S.L., Chen, Z. Du, Chen, F., Xiao, X.P., Lal, R., Zhang, H.L., 2015. Effects of tillage systems on soil organic carbon and total nitrogen in a double paddy cropping system in Southern China. Soil Tillage Res. 153, 161–168. https://doi.org/10.1016/j.still.2015.06.008

Zegada-Lizarazu, W., Monti, A., 2011. Energy crops in rotation. A review. Biomass and Bioenergy 35, 12–25. https://doi.org/10.1016/j.biombioe.2010.08.001

3 SOYBEAN-MAIZE SUCCESSION FITS SUCCESSFULLY TO CERRADO: BALANCING SOIL CARBON AND NITROGEN STOCKS AND CROP DIVERSIFICATION

Abstract

The land-use change (LUC) for agrosystems in Brazilian savannah (Cerrado) has resulted in degradation of soil C and N pools although conservation management practices adopted for soybean and maize production systems (SMPS) has been attempting to rebuild them. Crop diversification through rotation with legumes and grasses in SMPS has potential to enlarge the soil C and N stocks in alignment with N additions. This study investigated the effects of a nine-years experiment under SMPS at double cropping (DC) and crop rotation (CR) under N fertilizer levels on soil C and N pools. The soil was sampled down to 1.0 m for analyses of C and N forms (dissolved and total stocks); the topsoil was further evaluated for CO_2 evolution in a 500-d incubation assay. Additional soil samples from native vegetation (NV) were analyzed as reference. The conversion of NV to SMPS lead to a soil C and N depletion stressed at 0-0.2 m layer for total stocks and throughout the entire soil profile for dissolved C and N stocks. Comparing the treatments under SMPS and N levels revealed that nine years were not enough to detect changes in total C and N stocks. However, the dissolved organic forms of C and N were higher for DC under lower N level. The effect of past-fire events on NV resulted in higher accumulation of dissolved inorganic N down to 1.0 m driven by nitrate while the highest N level of CR increased the nitrate stocks downward. The mineralization assay revealed that increasing N levels promoted a decreasing on active and slow pools and stimulated the decomposition constant of all pools. Although no differences on total soil C and N stocks were observed SMPS and N levels treatments, the long-term mineralization assay indicates that the effects on field experiment might occur for longer periods. Overall, soybean-maize succession (DC) is a successful grain production system providing two profitable harvesting every year while maintaining soil C and N stocks like those observed in more diverse crop rotation systems.

Keywords: Land-use change; Crop rotation; Double cropping; Carbon mineralization; Dissolved stocks; Soil depth

3.1 Introduction

Brazil is the major player in global agricultural trade and the third largest exporter of agricultural products in the world (FAO, 2018). There are expectations that Brazil will present the largest increase in outputs than any other country by 2050 (Alexandratos and Bruinsma, 2012). Among the Brazilian biomes, the savannah (Cerrado biome) has a greater potential for agricultural production than other large scale producers such as North America, Europe, Argentina and Australia (Spehar, 2006).

The Cerrado biome in central region of Brazil covers 204 M ha (23% of the country) and its importance regarding global food security has been reached greater magnitude in the early of this century (Hosono et al., 2016). While prevailing naturally unfertile and acidic soils, the Cerrado region shifted from a *non-fit agricultural land* in the beginning of the 1960's to a current *major global breadbasket* due to its favorable edaphoclimatic conditions (i.e. soil structure and depth, topography, rainfall, temperature) (Hosono and Hongo, 2016a, 2016b). From 2002 to 2013 croplands under Cerrado region expanded from 21.6 to 24 M ha mainly over degraded pasturelands (INPE, 2015; Sano et al., 2010). In addition, the potential for expansion is even higher if considered that 38 M ha of degraded pasturelands are suitable for conversion to croplands (Rausch et al., 2019). The conversion of savannah vegetation to degraded pasture is a matter of international policy (Strassburg et al., 2014) since this land-use change (LUC) leads to water and soil degradation, causing negative effect on the chemical properties of soils and quality of streams (Hunke et al., 2015), in addition to reduction in soil C and N stocks following inadequate management practices such as overgrazing, no-fertilization, and no-liming (Batlle-Bayer et al., 2010). In opposite, the LUC from pastures to grain production under no-tillage with proper management practices and maintenance of soil fertility can recover the soil function as a sink of C and N (Batlle-Bayer et al., 2010; Bayer et al., 2006; Dieckow et al., 2009).

Crop rotation is an management practice that adds direct and indirect benefits to the agrosystem, such as increasing crop yield and soil biodiversity (Blanco-Canqui et al., 2015; Crusciol et al., 2015; Tiemann et al., 2015). In addition, crop rotation increases nutrient availability and cycling and may enlarge soil C and N stocks compared to lower-intensification agrosystems as mono- or double cropping (Tilman et al., 2002; Wittwer et al., 2017). The positive effect of crop rotation in soil C and N stocks occurs either for grasses or legumes species; while rotation with grasses has the potential to increase soil C stocks through C inputs of plant residues (Batlle-Bayer et al., 2010) legumes increases soil N stocks by biological fixation (McDaniel et al., 2014). Despite the well-known effects of crop diversification, in the Cerrado region still prevailing a grain production system based on double cropping of soybean (summer) followed by maize (fall) in succession. Whereas soybean-maize succession presents high profitability by allowing double-harvest per year, the lack of a more integrated crop diversification may affect soil ecology, reducing microbial diversity and increasing population of pests and plant nematodes (Grabau and Chen, 2016).

Increasing crop diversification through crop rotation, especially with grasses, raises questions such as the need of increasing N-fertilizers rates to avoid yield penalties caused by immobilization of N during the decomposition of rich C-residues. On this context, the effect of nitrogen fertilizer application is still in contest: while the addition of synthetic nitrogen fertilizers may favor soil C and N stocks by increasing biomass production and incorporation of crop residues (Riggs et al., 2015), excessive N application may favor microbial growth and the mineralization process of soil C and N (Chivenge et al., 2011; Kauer et al., 2015). Unbalancing N requirements is a challenge for agricultural systems since excessive N application resulted a decline in soil C (Khan et al., 2007) and N stocks (Mulvaney et al., 2009) in long-term experiments under temperate conditions.

Therefore, we evaluated a nine-years experiment under no-till in the Brazilian Cerrado aimed to investigate the long-term effects of soybean and maize production systems (SMPS) and N fertilizer levels on soil C and N stocks (total and dissolved forms). Also, we evaluated soil enzymes activity and carried out a long-term incubation assay for CO_2 evolution in topsoil samples (0-0.1 m) to clarify the effects of N inputs on the C and N mineralization processes. This study tested the hypothesis that: (i) crop rotation with grass and legumes is an effective management practice to increase soil total C and N stocks under SMPS compared to double cropping soybean-maize in succession, and; (ii) N fertilizer levels higher than crop's demand reduce soil total C and N stocks while increases their dissolved forms, mostly nitrate.

3.2 Material and methods

3.2.1 Site description and experimental design

The study was carried out in central region of Brazil, in the Cerrado biome, which is one of the main grain production area in the country. The study site was located at the Experimental Station Cachoeira (Fundação Mato Grosso) located at Itiquira city in the south region of Mato Grosso state (17°9'18,44"S, 54°45'15,32"W and 490 m; Fig. 1). According to Köppen-Geiger's system the climate is classified as Aw type or tropical wet-dry climate, which is characterized by rainfall concentrated in the spring and summer (October to April) while the dry season occurs in autumn and winter (May to September). The mean annual temperature is 24.5 °C and the annual precipitation is 1,260 mm (last 10-yr average).

In the study site, the conversion from savanna (Cerrado) to pasture occurred in the 1960's. However, it was not possible to identify the exact year of this land-use change (LUC). A second LUC converted pastures to croplands in 1992. In 2008, experiments under different grain production systems were established in the experimental station in which one of them was selected for this study. The experiment evaluated SMPS under no-till with different N fertilizer levels. Prior to its installing, a soil fertility analysis revealed that nutrient availability was suitable for grain production without need to correct its acidity according to the regional fertilization recommendation. In 2008, mechanical operations involved soil tillage with chisel plowing and heavy-disc harrowing up to 0.40 m depth. The experimental design was a randomized complete block under split-plot with four replications. Double cropping (DC) and crop rotation (CR) were applied to the main plots (49 m x 18.2 m; 892 m^2-size). DC involved the single succession of soybean as first crop followed by maize as second crop representing the main production system currently adopted in Cerrado (Corbeels et al., 2006; Maltas et al., 2007; Neto et al., 2010). CR involved a three-year rotation cycles with: soybean as first crop followed by legume as second crop in the first year; maize and grass sown together as first crop followed by remaining grass after maize harvest in the second year, and; soybean as first crop followed by maize and grass sown together as second crop in the third year. This three-year rotation system was repeated three times, totaling nine years of field activities. The experimental plots were triplicated to allow the harvest of soybean and maize in every single year (Fig. 2). All crops were harvested through machinery and their residues were deposited back on the soil surface. The second factor of the study was N fertilizer rates applied exclusively during the maize cultivation. Urea levels were top-dressed in the maize split-plots (223 m^2-size) as treatments of N fertilizer levels.

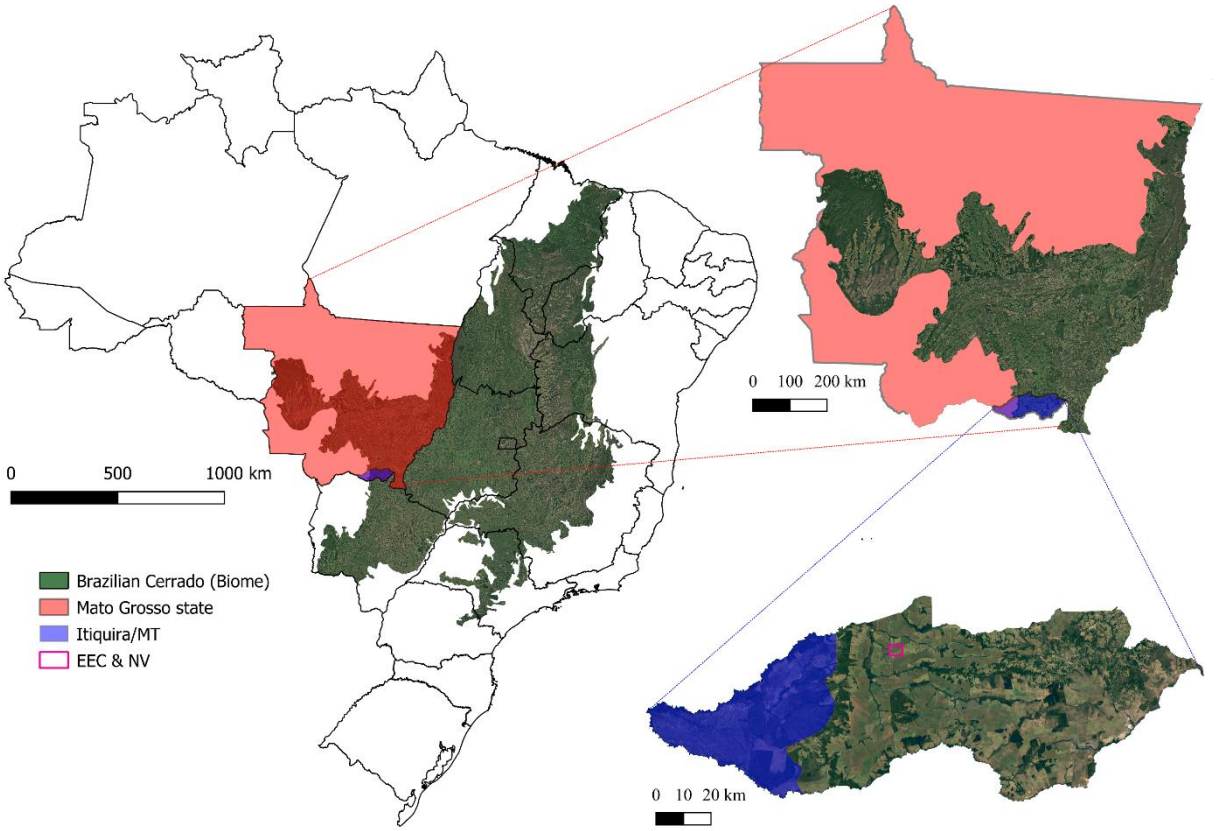


Fig. 1. Location of the study site in the south region of Mato Grosso state, central Brazil region

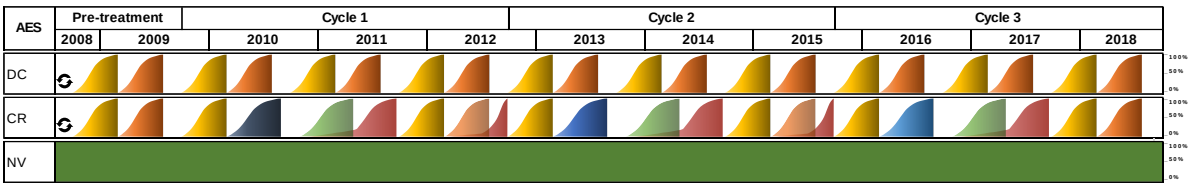


Fig. 2. Timeline of management practices and cultivation in each cropping system. AES: agroecosystems; DC: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; NV: native vegetation. , soybean growth cycle sown in October and harvested in February; , maize growth cycle sown in March and harvested in July; , maize growth cycle sown in November and harvested in April; , *Urochloa ruziziensis* growth cycle sown in November and desiccated in September; , *Urochloa ruziziensis* growth cycle sown in March and desiccated in July; , *Crotalaria spectabilis* growth cycle sown in March and desiccated in July; , *Crotalaria ochroleuca* growth cycle sown in March and desiccated in July; , *Cajanus cajan* growth cycle sown in March and desiccated in September; , undisturbed savannah wooded; , heavy-disc harrowing before experiment installation. The values on the right side are percentage of the biomass accumulation by crop

Long-term experiments are generally submitted to periodic updating in their study factors for the purpose of testing new concepts and hypotheses while keeping them relevant to current agricultural challenges (Calegari et al., 2008; Ferreira et al., 2013; Owens, 2013). Herein, at the end of each rotation cycle, maize and soybean cultivars were replaced by modern ones and the legume species were shifted in CR: *Crotalaria spectabilis* was adopted in the first rotation cycle, *Crotalaria juncea* in the second rotation cycle, and *Cajanus cajan* in the third rotation cycle (Fig. 2). Furthermore, other updates were carried out in the N top-dressing fertilization rates in maize along the rotation cycles. Larger N fertilization was adopted in the first-season maize rather than the second-season maize following official recommendations based on higher yield potential for first-season maize. In the first rotation cycle, the N rates adopted for the second-season maize were 0, 30, 60, and 90 kg ha^{-1} N and risen to 0, 40, 80, and 120 kg ha^{-1} N in the second and third rotation cycles (Table 1). For the first-season maize, the rates of 0, 70, 140, and 210 kg ha^{-1} N were adopted in the first and second rotation cycle, while the single rate of 210 kg ha^{-1} N was adopted in the third rotation cycle. Both the first- and second-season maize were fertilized at V4 stage following official recommendation and farmers' practice. Thus, due to the changes in N rates and differences in the N demand among maize seasons, we presented the N levels as: no N application (N1), lower N level (N2), moderate N level (N3) and higher N level (N4).

To investigate LUC effects, a representative native vegetation (NV) of native savanna was chosen ~4.1 km apart from the experimental site as reference area and four pseudo-plots (~600 m^2-size) were delimited ~50 m apart each other. The soils from both area were analyzed for physicochemical characterization (Table 2) and classified as Typic Haplustox (Soil Survey Staff, 2014), typical from Cerrado biome, which is highly weathered, with a predominance of the 1:1 clay mineral (kaolinite), iron oxides (goethite and hematite) and aluminum oxide (gibbsite) in the clay-size fraction (Coelho, 2019).

Table 1. Nitrogen (N), phosphorus (P) and potassium (K) levels (kg ha^{-1}) applied to crops over the cycles

Crops	Pre-treatment			Cycle 1			Cycle 2			Cycle 3		
	N	P	K	N	P	K	N	P	K	N	P	K
Soybean	0	60	30+(70)	0	45	(90)	0	45	(90)	0	45	(90)
Maize 1	30	40	50	30+(0/50/100/150)	40	60	30+(0/70/140/210)	40	60	30+(210/210/210/210)	76	60

Maize 2	30	40	50	30+(0/30/60/90)	40	40	30+(0/40/80/120)	40	40	00+(0/40/80/120)	40	40

Maize 1: maize sown in November and harvested in April; Maize 2: maize sown in March and harvested in July; values inside brackets represents the amount of nutrients applied as top-dressing, while the remaining values are the amount of nutrients applied at sowing; /: nitrogen levels applied as treatments (top-dressing).

Table 2. Physical and chemical soil characteristics from 0 to 1.0 m depth of the experiment station and at a representative undisturbed native vegetation

Depth	pH	ΔpH	OC	P	Ca	Mg	K	Al	H+Al	CEC	BS	m	Sand	Silt	Clay
m	H_2O		g kg^{-3}	mg kg^{-3}	mmol$_c$ kg^{-3}						%		g kg^{-1}		
ESC															
0-0.1	5,7	-1.1	22,6	14	32	14	1,4	4	75	122,4	39	8	294	72	634
0.1-0.2	5,9	-1.4	19,7	10	18	8	1,6	4	67	94,6	29	13	270	63	667
0.2-0.4	5,7	-1.1	18,6	7	15	7	1,3	2	58	81,3	29	8	263	90	647
0.4-0.6	5,6	-0.8	9,3	*2	9	3	1,0	1	38	51,0	25	7	240	55	705
0.6-0.8	5,7	-0.6	8,7	*2	9	3	0,8	*1	31	43,8	29	-	235	61	705
0.8-1.0	5,9	-0.6	9,3	*2	9	3	0,7	*1	24	36,7	35	-	241	39	720
NV															
0-0.1	4,4	-0.5	31,3	3	6	1	1,1	32	114	122,1	7	80	298	66	636
0.1-0.2	4,9	-0.9	26,1	*2	1	1	0,9	22	111	113,9	3	88	282	88	630
0.2-0.4	5,2	-1.0	18,6	*2	1	1	0,5	12	50	52,5	5	83	258	99	643
0.4-0.6	4,9	-0.6	13,9	*2	1	*1	*0,5	5	57	58,3	3	72	231	70	699
0.6-0.8	4,5	0.1	11,6	*2	1	*1	*0,5	2	50	51,3	4	51	246	81	673
0.8-1.0	4,4	0.4	9,3	*2	1	*1	*0,5	*1	42	43,2	4	-	249	76	675

ESC: experimental station Cachoeira; NV: native vegetation; ΔpH: pH 1 KCl – pH H_2O, indicating the net balance of exchangeable charges; OC: organic carbon; H+Al: potential acidity; CEC: cation exchange capacity at pH 7.0; BS: bases saturation; m: Al saturation; *: values below detection limit. All soil characteristics were analyzed as van Raij et al. (2001).

3.2.2 Soil sampling

At the end of the third rotation cycle, soon before maize harvesting (June 2018), disturbed soil sampling was performed in the experimental and NV areas respectively at each split-plot and pseudo-plot (plot) at 0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6, 0.6-0.8, 0.8-

1.0 m layers using a Dutch auger. Singles soil samples were taken into and between maize rows (*n* = 3 + 3 per layer per plot). In NV plots, sampling points were positioned in representative locations avoiding the surrounding ant/termite nests and root trees (*n* = 6 per layer per plot). These sampling positions represent the spatial variability within the plots so allowing a better estimation of chemical and enzymatic analysis. The singles samples were combined and mixed to obtain a composite soil sample for each layer and plot. In addition, undisturbed soil sampling was performed in the areas at each plot at 0.05, 0.15, 0.3, 0.5, 0.7, 0.9 m depth using stainless steel cores (~98 cm^3) to evaluate the soil bulk density whist representing the mean soil bulk density of each soil layer (0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6, 0.6-0.8, 0.8-1.0 m). Undisturbed soil samples were withdrawn into and between maize rows of DC and CR treatments, assuming no effect of N fertilization levels on soil bulk density (*n* = 1 + 1 per layer per plot). The soil bulk density results in the experimental plots were joint to obtain an average bulk soil density for each layer and plot. In NV plots, undisturbed soil samples were withdrawn assuming the same positioning and precautions taken in the disturbed sampling (*n* = 2 per layer per plot). Thus, the bulk soil density results in the plots were used to calculate soil C and N stocks (total and dissolved forms) throughout the soil profile.

In the laboratory, the composite soil samples were split in fresh and dried soil for respective evaluation of (i) dissolved C and N content, enzyme activity and CO_2 evolution, and; (ii) total C and N content. This splitting was proceeded due to different soil conservation requirements for each soil assessment. For the first measurements, fresh soil samples were stored at 4 °C to preserve the microbial activity; all coarse organic material were hand-picked and the samples were ground to pass through a 2.0 mm-sieve prior to analyses (Wollum, 1994; Zibilske, 1994). For the second measurements, soil samples were oven-dried at 40 °C for 72 h (Haney et al., 2004), and ground to pass through a 0.149 mm-sieve (Nelson and Sommers, 1996). Furthermore, enzyme activity and CO_2 evaluations were proceeded only on topsoil samples (0-0.1 m).

3.2.3 Physicochemical and enzymatic analyses

The soil total C and N content was analyzed in duplicate by dry combustion method using automated commercial instruments (CN628, LECO Corp., EUA). Before combustion some drops of 4 M HCl was added to aliquots of soil samples to confirm absence of carbonates. The analysis involved the complete combustion of all organic

matter in 100-mg of dried soil under pure O_2 at >1050 °C, followed the determinations of C and N respectively by CO_2 infrared detection and N_2 thermal conductivity (Nelson and Sommers, 1996).

Soil samples were extracted at room temperature (22 ºC) by horizontally shaking 5 g of fresh soil and 0.5 M K_2SO_4 at a 1:5 w/v ratio for 60 min at 180 rpm, followed by the soil suspension filtering (Jones and Willett, 2006). The soil extracts were readily analyzed for ammonium, nitrite + nitrate, and the dissolved C and N forms. Briefly, ammonium content was entirely determined into 96-well microplates by the salicylate–hypochlorite method, followed color reading at 667 nm (Mulvaney, 1996). Nitrite + nitrate content (nitrate) was entirely determined into 96-well microplates by acid vanadium reduction of nitrate to nitrite and further Griess–Ilosvay reaction, followed color reading at 540 nm (Miranda et al., 2001). Total dissolved nitrogen (TDN) analysis consisted by alkaline persulfate oxidation of soil extracts into glass tubes with screw caps at 1:1 v/v ratio under autoclave at 120 °C for 30 min, followed by nitrate determination above mentioned (Cabrera and Beare, 1993). Dissolved organic carbon (DOC) was analyzed by acid dichromate oxidation of soil extracts into glass tubes with screw caps at 1:1.2 v/v ratio under autoclave at 120 °C for 60 min, followed color reading into 96-well microplates at 590 nm (Islan and Weil, 1998). The colorimetric determinations were performed in duplicate using a microplate reader (Sunrise™, Tecan, Switzerland). Soil samples were oven-dried at 105 °C for 24 h to express the results on a dry basis (g kg^{-1}). From these results it was possible to quantify the dissolved forms of inorganic N (DIN) and organic N (DON). The DIN content was obtained as the sum of ammonium, nitrite and nitrate contents, while the DON content was calculated by difference of TDN and DIN contents.

Soil C and N stocks (total and dissolved forms) were obtained by equivalent soil mass method originally proposed by Ellert and Bettany (1995), which consist of correcting changes in soil bulk density using NV equivalent soil mass as reference. Since soil bulk density increase over time due to no-tilling practices, the bottom section of the topsoil mass was subtracted to ensure equivalent topsoil mass at NV. This removal section of topsoil mass was then accounted for the upper section of the second soil layer, and so on to the underlying layers (Lee et al., 2009). The sections thickness of the soil sampled layers and their stocks were calculated as follows:

$$Stu_i = \left\{L_i - \frac{[(Bd_{C.i} - Bd_{NV.i}) \times L_i]}{Bd_{C.i}}\right\} - Stb_{i-1} \quad \text{(Eq. 1)}$$

$$Stb_i = L_i - Stu_i \quad \text{(Eq. 2)}$$

$$SSeq = \frac{[(Stu_i \times C_i \times Bd_{C.i}) + (Stb_i \times C_{i-1} \times Bd_{C.i-1})] \times 10^4}{10^3} \quad \text{(Eq. 3)}$$

where *Stu* is the upper section thickness at the soil layer (m); *L* is the soil layer thickness (m); Bd_C is the changed soil bulk density (Mg m^{-3}); Bd_{NV} is the reference (NV) soil bulk density (Mg m^{-3}); *Stb* is the bottom section thickness at the soil layer (m); *SSeq* is the C and N stocks by equivalent soil mass (Mg ha^{-1}); *C* is the content of soil C and N (kg Mg^{-1}); 10^4 is the conversion factor from m^2 to ha; 10^3 is the conversion factor from kg to Mg of *C*, and; $_i$ is the ordinal number of the soil sampled layer. Ultimately, to better understanding the data the soil stocks were aggregated (sum) by layers obtaining accumulated soil stocks.

The enzymatic activity of β-D-glucosidase (BGC) and *N*-acetyl-β-D-glucosaminidase (BGN) were analyzed because they are soil enzymes linked to cellulose/cellobiose and chitobiose/chitin depolymerization towards the formation of glucose and glucosamine, respectively. These analyses were focused on topsoil (0-0.1 m) and subsurface (0.1-0.2 m) since enzymatic activity is known to decrease in depth. Briefly, 1 g of fresh soil samples were reacted with substrates buffered solution in water bath at 37 °C for 1 h, followed by the soil suspension filtering (Parham and Deng, 2000; Tabatabai, 1994). The soil extracts were readily analyzed by colorimetric determination of ρ-nitrophenol released by soil enzymes at 420 nm.

3.2.4 CO_2 evolution in long-term incubation assay

A long–term aerobic incubation assay was performed in the topsoil samples for assessing C and N mineralization. Briefly, 100 g of fresh soil from the 0-0.1 m layer was disposed into 600-cm^3 wide-mouth jar so that the soil volume was 100 cm^3. Soil moisture was adjusted to 50% of water-holding capacity (WHC) and left open in pre-incubation in the dark at 25 °C for 72 h to avoid initial flush of CO_2 biased by soil disturbances (e.g., handling). After the pre-incubation, a 80-cm^3 vial containing 0.5 M

NaOH standardized solution (CO_2-free) was trapped in a modified lid and then coupled to the jar, as the *diffusion system* built by Khan et al. (1997). The diffusion jars were kept in a dark room at 25 °C for incubation. Inside the closed jars, the released CO_2 diffuses into the NaOH solution during the incubation time. After this time, the vial was removed, some drops of 1.5 M $BaCl_2$ solution (CO_2-free) were added on it and the remaining OH^- was titrated to an end-point pH of 8.3 (Dilly, 2006) with 0.5 M HCl standardized solution (CO_2-free) assisted by automated commercial instrument (848 Titrino plus, Metrohm, Switzerland). While titrating the vials, the jars were left open for 1 h for soil gases exchange and to monitor their moisture. Distillated water was thoroughly dropped in the soil surface whenever the WHC decreased by 2.5%. The CO_2 emissions were measured after 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 21, 24, 28, 32, 36, 40, 44, 49, 54, 58, 65, 72, 79, 86, 93, 100, 107, 114, 121, 128, 135, 142, 149, 156, 163, 177, 191, 205, 219, 233, 247, 261, 296, 332, 359, 387, 422 and 500 d after the initial of the incubation. The volume of HCl dropped in the titration was used to calculate the amount of CO_2-C released during the incubation periods on oven-dry basis (g kg^{-1}) (Zibilske, 1994). The CO_2-C evolved were cumulated over time and rated by their respective total C content (g C kg^{-1}) to show the results on percentage as cumulative mineralized C from soil.

To describe the dynamics of soil C mineralization by microbial communities the cumulative data (%) were fitted in a first-order triple exponential decay model (triexponential mineralization pattern) to estimate the series of C pools and their decay constants (Motavalli et al., 1994). The model is described as follow:

$$C_{rem} = P_a \times exp^{-k_a \times t} + P_s \times exp^{-k_s \times t} + P_p \times exp^{-k_p \times t} \quad \text{(Eq. 4)}$$

where C_{rem} is the amount of C remaining in the soil at time t (%); t is the incubation time (d); P_a, P_s and P_p estimate the initial size of respective active, slow, and passive C pools (%); k_a, k_s and k_p estimate the decomposition constant of respective active, slow and passive C pools (d^{-1}). It was algebraically implicit that the parameters inputted in the triphasic model comprehend the discrete decay phases named as active (C_a), slow (C_s) and passive (C_p). Into this model, we considered that P_a and P_s are related respectively to C compounds of high to moderated lability as dissolved or particulate with no to low chemical or physical protection/stabilization while P_p is associated to

recalcitrant C fraction as “humified” compounds with high biochemical or physical protection/stabilization (Motavalli et al., 1994; Six et al., 2002).

3.2.5 Statistical analyses

The data residues were tested for normality distribution and homoscedasticity using Shapiro-Wilk’s test and Bartlett’s test ($p > 0.100$) respectively. The data from physicochemical and enzymatic analyses were inputted in a two-way ANOVA, where *SMPS* and *N fertilizer levels* were considered as fixed factors within the split-plot statistical model ($p < 0.100$). When the *F*-test showed significance, Tukey's HSD test was applied to identify the differences between the means of the treatments ($p < 0.100$). The NV treatment was not input in the statistical model due to its experimental design limitations but considered as reference values when comparing LUC. Regardless of significance of the *F*-test all the interactions were showed.

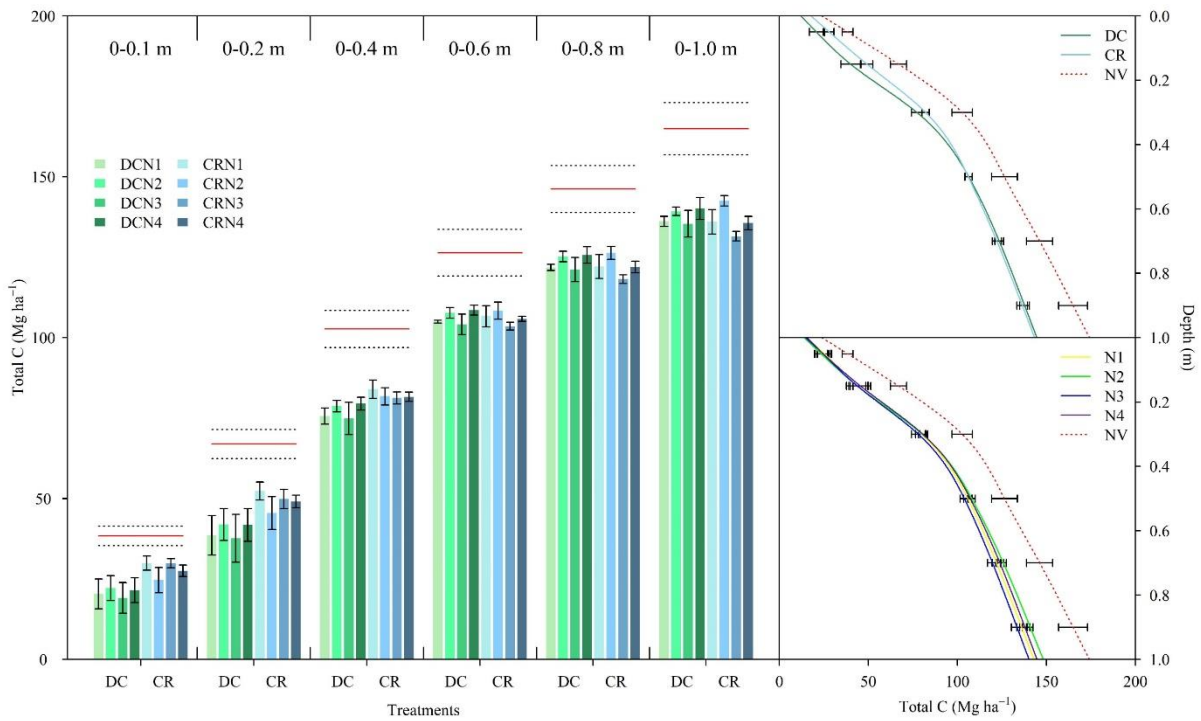
The data from CO_2 evolution (mg C kg^{-1} soil) in the long-term incubation assay were subtracted from the total C content (g C kg^{-1} soil) for each sample and the results showed as soil C remaining (%). The nonlinear regression described in Eq. 4 was fitted to the decaying data through the *nlme* function (Pinheiro et al., 2018) which involved a quasi-Newton method optimizer to estimate the initial parameters (P_a, k_a, P_s, k_s, P_p, k_p) by decreasing the mean square error to the minimum. A mixed-effect model was combined to the nonlinear regression fitting considering *SMPS*, *N fertilizer levels* or their interaction as *fixed* factors and *block* as *random* factor. The model fitting also considered the heteroscedasticity adjustment through exponential modeling of variance (*weights* argument) and correlation structure through temporal dependencies of the errors (*correlation* argument) whenever necessary. Following, a model reduction technique was proceeded for pairwise comparison in order to answer the treatments effects on the dynamics of soil C mineralization in each decay phase (C_a, C_s and C_p) and entirely (C_{rem}) (Ritz and Streibig, 2009). Briefly, a robust model including the data from all treatments was fitted and compared against a reduced model composed by combining pair of treatments (e.g., a + b + c + d + e *VS* ab + c + d + e; a + b + c + d + e *VS* a + bc + d + e) using the likelihood-ratio test ($p < 0.100$).

The data are presented as mean value followed by standard error of the mean (SEM). All statistical analyses and graphs were made in R (R Core Team, 2019).

3.3 Results

3.3.1 Soil C and N stocks in depth

Overall, the accumulated pattern of soil C and N stocks in soil depth were similar, as consequence of their distribution between each layer. After nine years of experiment, there were no differences between treatments in total C (TC) and total N (TN) stocks throughout the soil profile while the NV presented higher TC and TN stocks ranging from 38.3 and 1.9 Mg ha^{-1} at 0-0.1 m layer to 165 and 8.4 Mg ha^{-1} down to 1.0 m, respectively (Fig. 3). At upper layers, down to 0.2 m, the CR showed some initial detachment towards NV stocks as a difference of 9.2 and 0.6 Mg ha^{-1} on TC and TN stocks respectively as compared to DC. Conversely, at 0.2-0.6 m layer, the DC treatment presented increments of TC and TN stocks by 17 and 25% higher than CR, thus equilibrating the TC and TN stocks in deeply soil layers. On the other hand, it seemed that N fertilization acted in opposite way by incept to split the levels from each other below 0.4 m depth. The distribution of both TC and TN stocks in NV reference showed superiors stocks related to treatment average at top- and subsoil layers with increments of 19-52% for TC and 20-42% for TN stocks. Still, even a significant increasing of treatment average on TC and TN stocks between 0.2-0.6 m layer were not enough to surpass the stocks at NV accumulated below that layer (Fig. 3 and Suppl Fig. 1 of the Appendix).



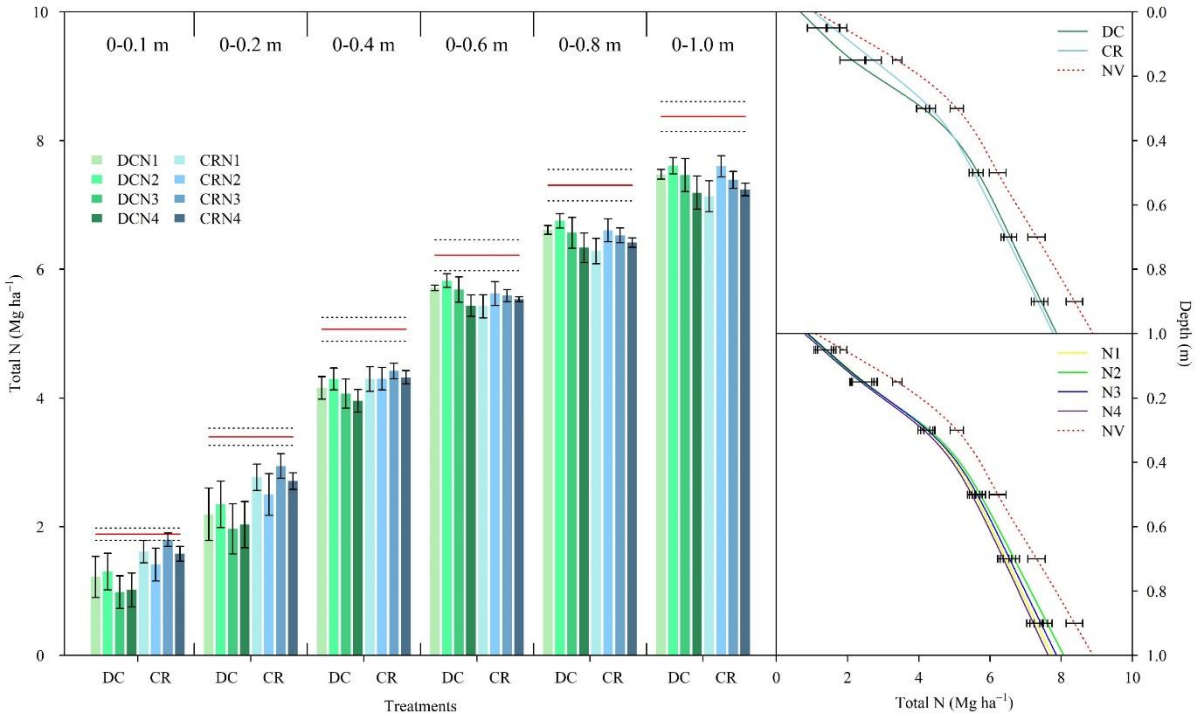


Fig. 3. Total C (TC) and total N (TN) stocks accumulated in soil depth as affected by treatments (left), SMPS (top right) and N fertilizer levels (bottom right). DC: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; N1: no N application; N2: lower N level; N3: moderate N level; N4 higher N level; NV: native vegetation. Columns and error bars represent the mean ± SEM. Red-solid lines and black-dashed lines indicate the mean ± SEM of the NV

The DIN was distinctly influenced by SMPS and N fertilization. In the case of accumulated ammonium stocks, no differences were found between treatments in any depth up to 1.0 m and its distribution followed the same pattern as in TN and TC stocks but a slightly increase of DIN occurred on the 0.2-0.4 m and 0.4-0.6 m layers (Suppl. Fig. 2 and 3 of the Appendix). The distribution of ammonium stocks was kept at low values among layers ranging from 2.9 to 10.5 kg ha^{-1} for SMPS treatments and from 6.5 to 14.8 kg ha^{-1} at NV. However, the distribution of nitrate stocks had trace values of down to 0.2 kg ha^{-1} mainly distributed at 0.6-1.0 m layer for SMPS treatments while high stocks of up to 72.6 kg ha^{-1} observed at NV considering the same layer. The CR presented effects on accumulated nitrate with higher stocks mainly in CRN4 from 0-0.4 to 0-1.0 m caused by an increasing on nitrate in 0.2-0.4 and 0.4-0.6 m layers (Suppl. Fig. 2 and 3 of the Appendix). The sum of ammonium and nitrate as accumulated dissolved inorganic N (DIN) mitigated all the interaction effects on it but CR and N4 were maintained higher than CS and others N levels from 0-0.4 m to 0-0.1

m (Fig. 4). Also, the ammonium and nitrate relations were converse between SMPS treatments and NV. The proportion of ammonium on DIN was higher than nitrate and further increased from 62 to 74% towards depth layers in SMPS treatments while the NV showed higher proportion of nitrate on DIN from 56 to 78% (Suppl. Fig. 4 of the Appendix). The DIN stocks reached 232.4 kg ha^{-1} accumulated on 0-0.1 m at NV, about four-fold more the DIN stocks obtained in SMPS treatments (Fig. 4).

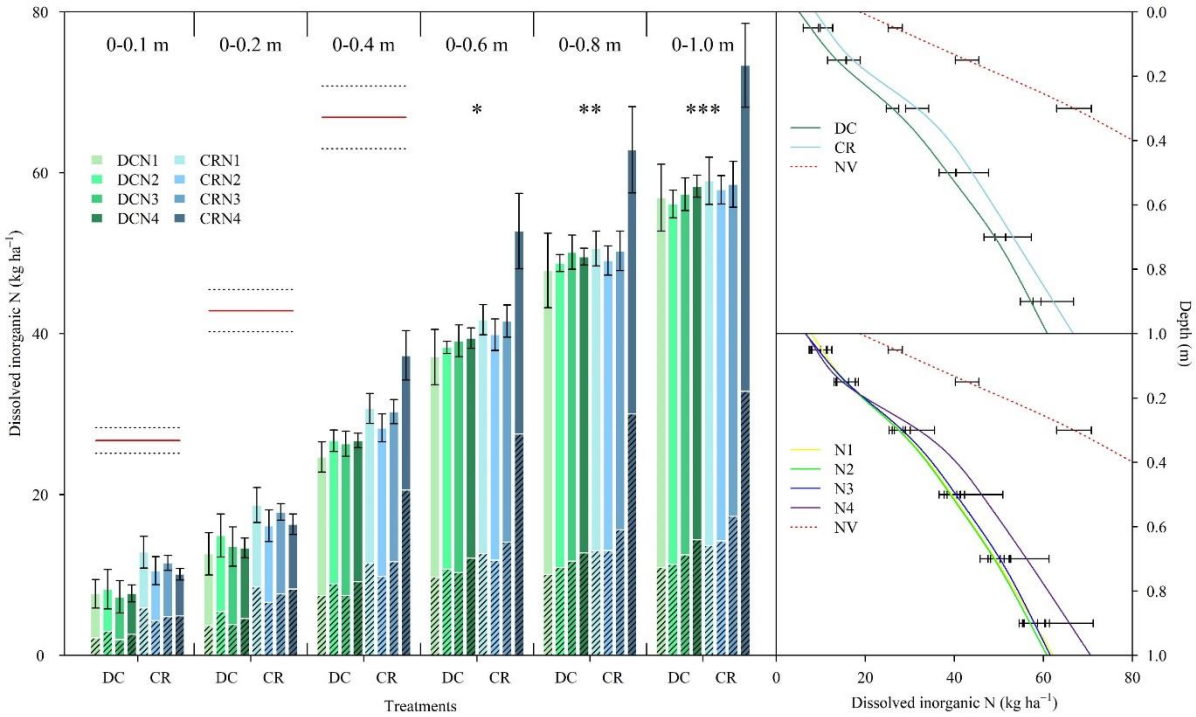


Fig. 4. Dissolved inorganic forms of N stocks accumulated in soil depth as affected by treatments (left), SMPS (top right) and N fertilizer levels (bottom right). DC: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; N1: no N application; N2: lower N level; N3: moderate N level; N4 higher N level; NV: native vegetation. Columns and error bars represent the mean ± SEM of DIN and dashed columns the mean of nitrate. Red-solid lines and black-dashed lines indicate the mean ± SEM of the NV. *, ** and *** respectively represent the NV mean ± SEM of DIN stocks of 96.4 ± 6.9, 153.1 ± 8.9 and 232.4 ± 11.0 Mg ha^{-1}

In agreement to the inorganic N forms, the accumulated DON and DOC stocks of SMPS treatments were closer to NV in topsoil but spread to wide differences down to 1.0 m (Fig. 5). The differences between SMPS treatments for DON were diminished as subjacent layers were accounted for (accumulated effect) while DOC was similar in all treatments. The dissolved organic forms of C and N had similar distribution on soil depth following the previous found in TC and TN stocks mainly in SMPS but without any difference between DC and CR of maximum variation of 61.2 and 2.2 kg ha^{-1} for DOC and DON, respectively. Comparing N levels, the dissolved organic forms showed some grouping as far from the top with highest stocks for N1 and N2 and lowest for N3 and N4 (Fig. 5). Interestingly, the DOC/DON ratio of SMPS and N levels were consistently around the NV from top- to subsoil layers (data not showed).

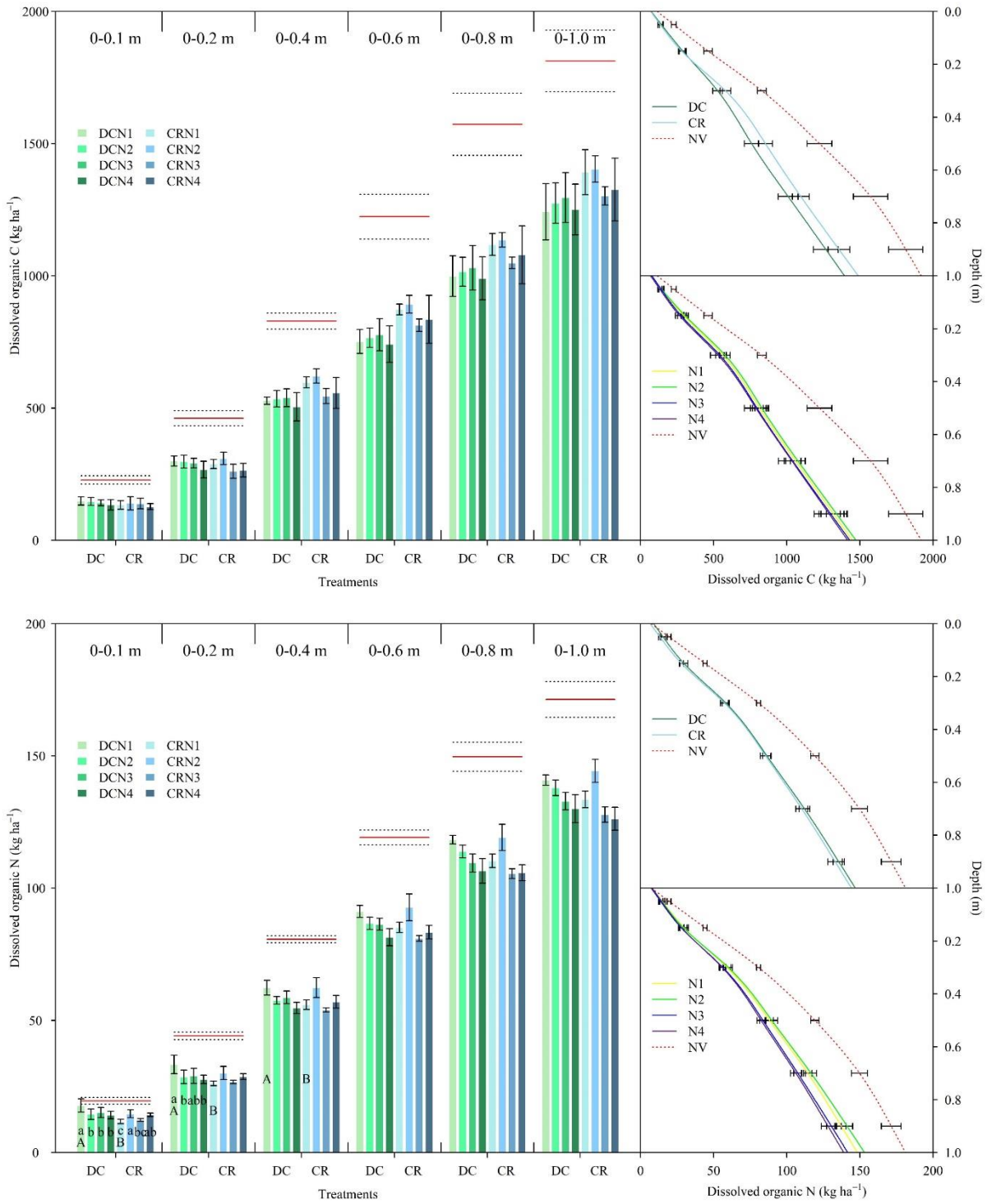


Fig. 5. Dissolved organic N (DON) and C (DOC) stocks accumulated in soil depth as affected by treatments (left), SMPS (top right) and N fertilizer levels (bottom right). DC: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; N1: no N application; N2: lower N level; N3: moderate N level; N4 higher N level; NV: native vegetation. Columns and error bars represent the mean ± SEM. Red-solid lines and black-dashed lines indicate the mean ± SEM of the NV. * represent the mean ± SEM of the NV of 232.4 ± 11.0 Mg ha^{-1}. Lowercase and capital letters into each column respectively indicate differences among treatments at N fertilizer level inside SMPS and at SMPS inside N fertilizer levels, according to Tukey's test (p < 0.100)

3.3.2 Soil C mineralization kinetics and enzymatic activities

Soil C mineralization was < 6% of the total C content after 500 d of incubation, but wide enough for detection of differences among treatments for CO_2 release and its decay kinetics. On average, the total CO_2-C evolved from DC and CR were 1.16 and 1.33 g kg^{-1}, respectively, while NV reached 2.58 g kg^{-1}. In addition, the N fertilization decreased the C emissions from 1.31 to 1.19 g kg^{-1} as N level increases from N1 to N4. The accumulated emissions from DC, CR and NV were proportional to their total C contents (r = 0.89; $p < 0.001$). Although total emissions of CO_2-C in CR were 14.7% higher than DC, 95.92% of the total C content was maintained in CR against 95.06% in DC. However, the lower CO_2-C emissions evolved from N fertilization as N3 and N4 were followed by an increasing on soil C conservation on average of 95.3% against 95.6% from N1 and N2 (Table 3).

Table 3. Total CO_2-C evolved, C_{rem} and soil organic C (SOC) as affected by treatments under SMPS and N fertilizer levels

Treatment	Total evolved	C_{rem}	SOC
	g CO_2-C kg^{-1} soil	%	g C kg^{-1} soil
DC	1.16 ± 0.08	95.06 ± 0.17 b	23.8 ± 2.2
DCN1	1.19 ± 0.20	94.66 ± 0.39 bB	23.3 ± 5.3
DCN2	1.23 ± 0.21	95.13 ± 0.09 ab	25.3 ± 4.4
DCN3	1.14 ± 0.17	94.93 ± 0.35 abB	21.8 ± 5.5
DCN4	1.06 ± 0.12	95.50 ± 0.38 a	24.6 ± 4.4
CR	1.33 ± 0.05	95.92 ± 0.13 a	32.0 ± 1.4
CRN1	1.44 ± 0.04	96.02 ± 0.17 abA	34.3 ± 2.5
CRN2	1.24 ± 0.14	95.47 ± 0.32 b	28.2 ± 4.8
CRN3	1.33 ± 0.08	96.39 ± 0.07 aA	34.2 ± 1.7
CRN4	1.32 ± 0.08	95.79 ± 0.14 ab	31.5 ± 2.1
NV	2.58 ± 0.10	94.01 ± 0.26	43.8 ± 3.5

Values represent the mean ± SEM. DC: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; N1: no N application; N2: lower N level; N3: moderate N level; N4 higher N level; NV: native vegetation. Lowercase and capital letters besides in treatments values respectively indicate differences among treatments at N fertilizer level inside SMPS and at SMPS inside N fertilizer levels, according to Tukey's test ($p < 0.100$). Lowercase besides in SMPS average indicate differences among them, according to Tukey's test ($p < 0.100$).

The mineralization kinetic through modelling the soil CO_2-C evolution in a multiphase pattern of first-order exponential decay reactions was previously fitted for both biphasic and triphasic decomposition and the results showed better fitting for the

triple one ($p < 0.001$; data not showed). The likelihood (L) of the models fit were 1485, 1514 and 1674 involving each of the factors SMPS, N fertilizer levels and their interaction respectively while 62 apart for the single NV and showed better fit by interaction (Table 4). The sum of the three pools into the models was on average 99.96 ± 0.01%. Considering the pools partition into the models the treatments had similar proportion between P_a, P_s and P_p that varied from 0.25, 1.25 and 96.53% to 0.57, 3.09 and 98.35%, respectively, while the NV had the lowest values for active (0.23%) and passive (97.28%) pools. The correlation (r) between DOC contents and P_a was 0.41 ($p = 0.013$).

Table 4. Effect of treatments under SMPS and N fertilizer levels on parameter and likelihood values for triple exponential decay model describing the soil C mineralization dynamics

Treatment	ln(L)	P_a (%)	k_a (d^{-1})	P_s (%)	k_s (d^{-1})	P_p (%)	k_p (d^{-1})
Reference	62						
NV		0.23 ± 0.04	0.32 ± 0.13	2.48 ± 0.32	0.006 ± 0.001	97.28 ± 0.34	0.00007 ± 0.000008
SMPS	1514						
DC		0.49 ± 0.03	0.11 ± 0.01	1.57 ± 0.06	0.010 ± 0.001	97.84 ± 0.09	0.00006 ± 0.000003
CR		0.31 ± 0.02	0.29 ± 0.04	2.21 ± 0.10	0.008 ± 0.001	97.46 ± 0.12	0.00003 ± 0.000003
N levels	1485						
N1[@]	1484	0.47 ± 0.04	0.11 ± 0.02	2.05 ± 0.17	0.008 ± 0.001	97.38 ± 0.20	0.00004 ± 0.000006
N2[@]	1484	0.42 ± 0.04	0.13 ± 0.02	2.09 ± 0.15	0.008 ± 0.001	97.41 ± 0.18	0.00004 ± 0.000005
N3[#]	1485	0.33 ± 0.03	0.32 ± 0.07	1.66 ± 0.08	0.010 ± 0.001	97.98 ± 0.10	0.00005 ± 0.000005
N4[#]	1485	0.34 ± 0.03	0.33 ± 0.07	1.70 ± 0.08	0.010 ± 0.001	97.95 ± 0.10	0.00005 ± 0.000005
SMPS:N levels	1674						
DCN1[$]		0.37 ± 0.04	0.54 ± 0.13	1.57 ± 0.04	0.018 ± 0.001	98.06 ± 0.06	0.00007 ± 0.000004
DCN2[&]	1671	0.57 ± 0.07	0.08 ± 0.01	2.13 ± 0.35	0.006 ± 0.001	97.19 ± 0.40	0.00004 ± 0.000008
DCN3[&]	1671	0.43 ± 0.04	0.17 ± 0.04	1.76 ± 0.11	0.010 ± 0.001	97.73 ± 0.14	0.00006 ± 0.000005
DCN4[§]		0.38 ± 0.04	0.38 ± 0.09	1.25 ± 0.06	0.013 ± 0.001	98.35 ± 0.08	0.00006 ± 0.000004
CRN1[&]		0.35 ± 0.04	0.25 ± 0.06	3.09 ± 0.43	0.005 ± 0.001	96.53 ± 0.45	0.00001 ± 0.000009
CRN2[$]	1669	0.36 ± 0.04	0.25 ± 0.06	2.20 ± 0.15	0.008 ± 0.001	97.42 ± 0.18	0.00004 ± 0.000005
CRN3[§]		0.25 ± 0.04	0.40 ± 0.14	1.64 ± 0.12	0.009 ± 0.001	98.10 ± 0.15	0.00004 ± 0.000005
CRN4[$]	1669	0.28 ± 0.04	0.32 ± 0.10	2.33 ± 0.18	0.008 ± 0.001	97.38 ± 0.20	0.00003 ± 0.000005

DC: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; N1: no N application; N2: lower N level; N3: moderate N level; N4 higher N level; NV: native vegetation. *ln(L)*: is the log transformation of the likelihood function that measure the goodness of the model fit, C_a: active pool size (%), k_a decay constant at active pool (d^{-1}), C_s: slow pool size (%), k_s decay constant at slow pool (d^{-1}), C_p: passive pool size (%), k_p decay constant at passive pool (d^{-1}). Values represent means ± SEM (n = 4) of the

measured replicates. All parameters were significant to their model fit at $p < 0.100$. Discrete symbols were used due to model's comparison in which [@] and [#] indicate significant differences into N levels main effect and [$], [&] and [§] indicate significant differences among N levels within systems. NV was separately modelled for comparison as reference. *ln(L)* values for Reference, SMPS, N levels and SMPS:N levels represent the likelihood for the model fitted without grouping treatments while the other values inside represent the likelihood as pair of treatments are grouped and tested for likelihood-ratio test at $p < 0.100$; blank cells have no equal group.

The model fitting by treatments revealed that the effect of N levels within SMPS affected the decomposition kinetics at discrete proportions by each parameter (Table 4) while were not visually distinguished by their C_{rem} decay patterns (Fig. 6). Increasing the N fertilization from N2 to N4 in DC and from N1 to N3 in CR promoted a decreasing of P_a and P_s and an increasing of P_p and the decomposition constants (k_a, k_s, k_p). The C mineralization kinetics showed no difference for N2 and N3 in DC and for N2 and N4 in CR with *L* of 1671 and 1669, respectively.

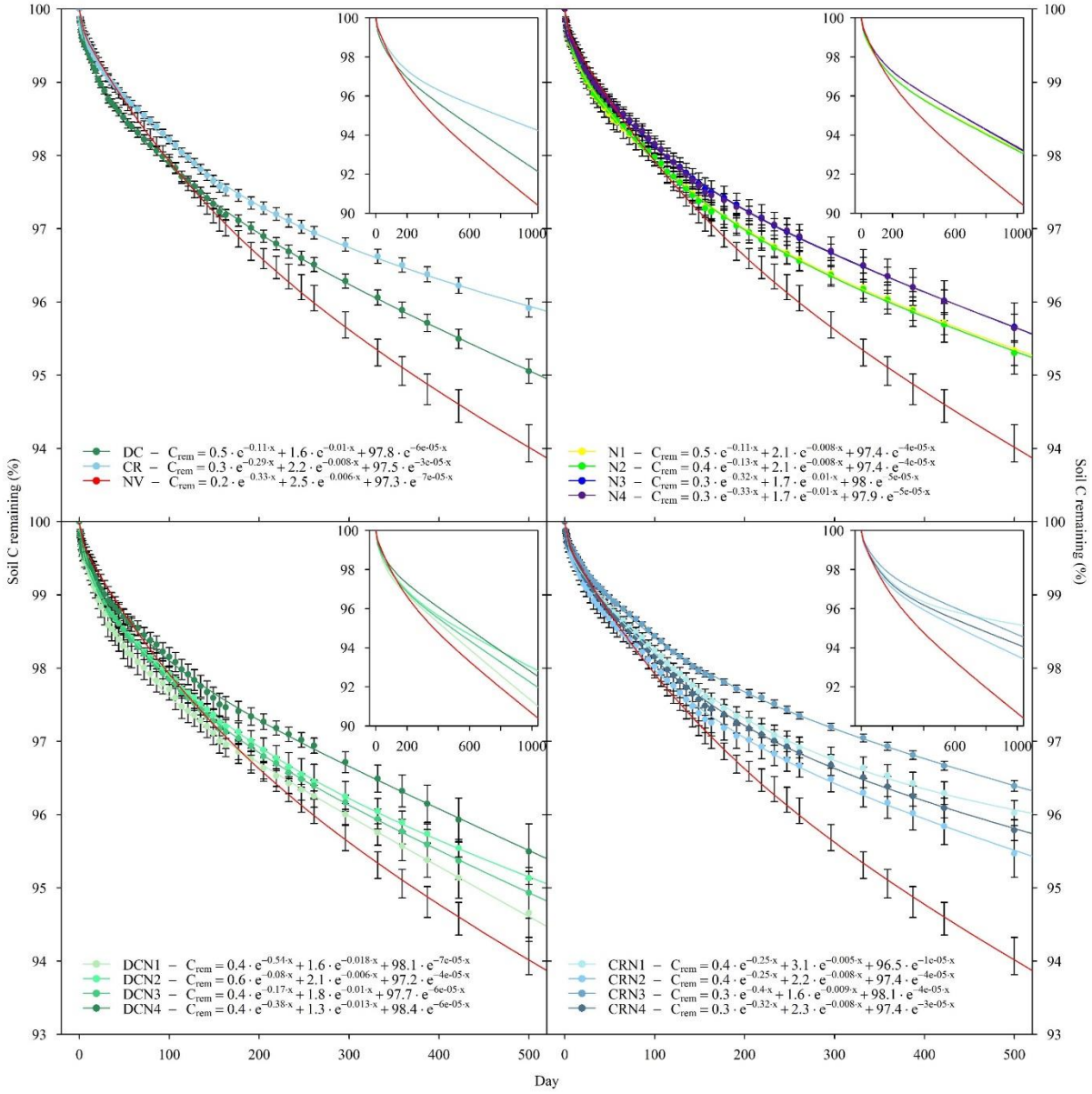


Fig. 6. The percentage of soil C remaining (%) during 500 d incubation at main factors SMPS and N fertilizer levels (top panels) and treatments (bottom panels). DC: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; N1: no N application; N2: lower N level; N3: moderate N level; N4 higher N level; NV: native vegetation. Full circles represent means ± SEM (SMPS n = 32, N fertilizer levels n = 32, treatments n = 4, NV n = 4) of the measured replicates and the triple exponential decay model were fitted by main factors or treatment based up on all replicates. The equations are shown on bottom of the panels and the statistics of their parameters are in Table 4. The inset graphs provide an estimation over 1000 d incubation

The model reduction detected that active decay phase could be considered equal for all treatments (*L* = 1664). However, considering N levels as factor, the models fitting had the lowest *L* (1485) but showed the major model reduction by grouping N1 and N2 (1484) as well as N3 and N4 (1485). This reduction clearly aggregated the N

fertilization onto groups of inferior (N1 and N2) and superior (N3 and N4) N levels and simplified the understanding of the effect of this factor on C mineralization that were the increasing on the decay constants of the three phases (k_a, k_s and k_p) and the decreasing of the pools with low to moderate lability (P_a and P_s) at the superior N levels. The SMPS strongly affected the mineralization kinetics as DC and CR had no decomposition phase shared between them while the model reduction detected that the passive phase was the most affected by that factor so decreasing the *L* from 1514 to 1220. Accordingly, the k_p of DC was double that of CR and closer to the highest decay for passive phase found at NV (Table 4 and Fig. 6).

No differences were found on soil enzymatic activities. In general, BGC and BGN activities in topsoil samples were two-fold higher than in subsurface except in BGC at NV. Also, the BGC and BGN activities had higher range in topsoil samples than subsurface varying from 48.8 to 81.0 mg kg^{-1}soil h^{-1} and from 39.6 to 67.7 mg kg^{-1} h^{-1} respectively in topsoil (Fig. 7).

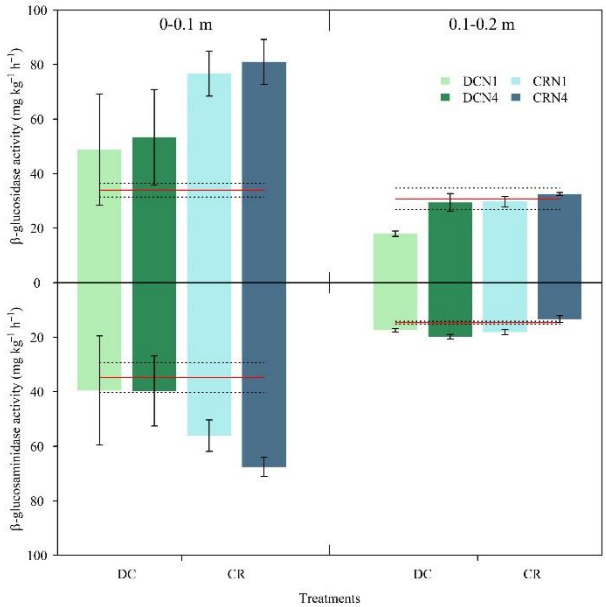


Fig. 7. Enzymatic activity of β-D-glucosidase β-D-glucosaminidase at 0-0.1 and 0.1-0.2 m as affected by treatments under SMPS and N fertilizer levels. DC: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; N1: no N application; N4 higher N level. Columns and error bars represent the mean ± SEM. Red-solid lines and black-dashed lines indicate the mean and ± SEM of the native vegetation (NV). Lowercase and capital letters into each column respectively indicate differences among treatments at N fertilizer level inside SMPS and at SMPS inside N fertilizer levels, according to Tukey's test (p < 0.100)

3.4 Discussion

3.4.1 LUC, SMPS and N fertilization on total C and N stocks

SMPS treatments followed a similar accumulation and distribution pattern of TC and TN stocks but in lower amounts as compared to NV (Fig. 3). The conversion of native tropical forest to agrosystems commonly lead to a soil C depletion (Don et al., 2011) mainly due to the usual soil disturbance and the successive harvesting of large fraction of commercial crops (Palm et al., 2014). The LUC to SMPS resulted in an average C loss of 27 Mg ha^{-1} accumulated up to 1.0 m over +50 years. Accordingly to Luo et al. (2010), ~75% of such C loss could be occurred in the first 5 years after conversion and the remaining 25% slowly decayed in a quasi-steady state (Murty et al., 2002). Aligned to C losses, TN stocks followed the same pattern of depletion throughout the soil profile as already expected due to the ecological principle of nutrient stoichiometry of soil organic matter (i.e. mainly C, N, P, S) (Cleveland and Liptzin, 2007; Hessen et al., 2004; Kirkby et al., 2011; Sinsabaugh and Follstad Shah, 2012). In this study, the proportional losses of C and N through LUC were caused by soil disturbance and low nutrients inputs in SMPS that ultimately promoted organic matter decomposition with release of CO_2-C to atmosphere and exportation of N, P and S through the harvested grains. The C:N ratio of the soil in the agrosystem evaluated herein was not responsive to LUC (data not shown) as found in a comparative analyzes in Brazil by Zinn et al. (2018).

In Cerrado region, warm temperatures and seasonal precipitation over highly weathered soils boost the decomposition of plant residues and soil organic matter. The cultivation of two crop species per year, besides increasing profitability, is a mean to increase biomass production (above and belowground residues) which might help to sustain SOC levels higher than in monoculture areas. The double cropping of soybean followed by maize – as second commercial crop – in Cerrado, also known as soybean-maize succession, has been known since 1980s as an alternative agrosystem to soybean monocropping with effective advantages over land conservation, nutrient cycling, increasing yield and cost savings (de Freitas and Landers, 2014; Scopel et al., 2013). Introducing crop rotation through diversification of both commercial and cover crops has advantages towards enhancing soil physical and biological quality (Zegada-Lizarazu and Monti, 2011). However, into the conservation agriculture, the effect of crop diversification onto C and N sequestration through crop rotation may be considered less apparent than the other two principles of suppression or reducing

tillage and maintaining a covered soil with biomass (alive or residues) (Palm et al., 2014). Also, the effects of crop rotation on soil C and N stocks can be considered mixed (Luo et al., 2010; McDaniel et al., 2014; West and Post, 2002). Conversely to our hypothesis (i), after nine years experiencing crop diversification the CR was not a management practice able to increase soil total C and N stocks in SMPS compared to DC. The results demonstrate that TC and TN accumulated stocks showed no differences between treatments at any layer, even obtaining significant differences on average both for C inputs of 8.6 and 9.6 Mg ha^{-1} yr^{-1} and N inputs of 209.0 and 266.7 kg ha^{-1} yr^{-1} (N fertilization not accounted) between DC and CR, respectively, over the nine years of experiment (data not shown). Considering the residues inputs that have been maintained since 2010, our data suggest that either the treatments effect on soil C and N stocks requires more time to show up, or the soil evaluated herein is already approaching the steady-state level. Both suggestions are widely supported by literature (Batlle-Bayer et al., 2010; McDaniel et al., 2014; Poeplau and Don, 2015; Santos et al., 2011; Stewart et al., 2007; West and Post, 2002) and future studies on this site could state this issue.

Conversely to our hypothesis (ii), higher N fertilization levels did not decrease soil TC and TN stocks after nine years. We could even expect a rising on TC and TN stocks under N fertilization levels considering that the inputs of C and N through crop residues ranged from 8.5 to 9.7 Mg ha^{-1} yr^{-1} and from 211.0 to 254.6 kg ha^{-1} yr^{-1}, respectively (N fertilization not accounted) (data not shown). Indeed, few studies have been addressing the N fertilization effects on Cerrado agrosystems because most of them are under N-fixing soybean croplands that requires little or no N additions (Rausch et al., 2019). However, the increasing interest on agricultural intensification has been led to the introduction of other cash crops in succession with soybean, mainly maize and cotton, that hold high N demand supplied by N fertilizer and may affect the soil C and N dynamics (Brando et al., 2013; Hunke et al., 2015). In our study, the N fertilization levels were applied in V4 stage of maize looking for its yield response and the data showed that the N fertilizer increased not only grain yield, but also N content in the grain, N output from grain harvesting and C and N inputs from residues for maize, whereas no beneficial effects of N residual were detected for soybean (data not shown). These results highlight that the N fertilization effect on C and N inputs accumulated along nine years came mostly from maize crop.

3.4.2 LUC, SMPS and N fertilization on dissolved C and N stocks

There was a significant reduction in the dissolved inorganic N stocks, mainly nitrate, from the NV to the CR and DC (Fig. 4). Actually, soils under native vegetation in Cerrado, especially represented by Oxisols, have naturally low fertility due to nutrient leaching and acidification throughout the weathering process (Goedert, 1983; Hunke et al., 2015). These soils frequently present high content of Fe and Al-oxides that hold pH-dependent charges. At deep layers the organic matter is scarce and the soil pH may descend the point of zero charge resulting a net positive balance of charge that enhance anions adsorptive capacity, like nitrate (Atkinson, 1967; Parks and De Bruyn, 1962; Yopps and Fuerstenau, 1964). Although we had not directly measured the point of zero charge of soils, the net balance of exchangeable charges might be estimated by the difference between the pH value determined at 1M KCl and H_2O, also known as Δ pH (Mekaru and Uehara, 1972). The soil chemical characterization of NV (Table 2) showed low base saturation (3-7%) and high aluminum saturation (51-80%) throughout the profile besides of strong acidification (pH 4.4-5.2) mainly below 0.6 m where the Δ pH was positive in alignment with the pronounced stocks of nitrate. However, the soil under SMPS had its properties changed probably due to management practices of lime and gypsum application over the past 50+ years printing their effects even down to one meter where a weak acidification was detected, possibly presenting a net negative balance of charge. Although we can explain the distribution of dissolved inorganic species in the soil profiles supported by the alteration of the net balance of exchangeable charges, a high amount of nitrate in soil depth under Cerrado vegetation is not normally found in literature (Lilienfein et al., 2003; Wilcke and Lilienfein, 2005) but converse since Cerrado is commonly known as a N-limited ecosystem characterized by presenting low rates of nitrification in dominance of ammonium in soil (Bustamante et al., 2006, 2004). We therefore believe that the high nitrate stocks in depth could be caused by past fires. After a fire event in the dry season, the nitrate in the ashes left in the soil surface solubilize along the following rainy seasons that may reach down to 2.0 m depth in the soil solution (Oliveira-Filho et al., 2018). Nevertheless, the nitrate leached could be cycled since large portion of roots are commonly found down to 1.0 m for water uptake during the dry season (Jackson et al., 1999; Quesada et al., 2008).

The LUC towards agrosystems promoted a decreasing in the DOC and DON stocks both accumulated and distributed throughout the soil profile (Fig. 5). LUC

studies at Cerrado also found a reduction of the dissolved organic matter (i.e. DOC, DON) fluxes in the soil layers after conversion from native vegetation to second forests (i.e. pine, eucalyptus) and pasturelands (Ciglasch et al., 2004; de Brito et al., 2019). In soils under native vegetation in Cerrado, the organic material in the superficial and underlaying horizons composed respectively by leaves left by decidual trees during the dry season and root exudates supply nutrients (i.e. C, N, P) for the decomposition process at high rates that produce DOC and DON (Ciglasch et al., 2004; Kalbitz et al., 2000; Michalzik et al., 2001). This process was also reveled in our mineralization assay. The rates and downward fluxes above mentioned might be even increased in the beginning of the rainy season as consequence of combination of edaphoclimatic factors as intense rainfall, warm temperatures and high hydraulic conductivity of soils (Hunke et al., 2015; Kalbitz et al., 2000; Lilienfein et al., 2003). The dissolved organic matter also presents net charges and polarity that may favor or not its adsorption/retention to mineral surfaces or outfluxes through mineralization and leaching (Kalbitz et al., 2000; Marschner and Kalbitz, 2003). In acid forest soils, like in this study, the availability of Al in solution reacts with the dissolved organic matter forming metal complexes that may either reduce its mineralization due to toxicity and inhibition of enzyme activity (Schwesig et al., 2003) or increase its solubility by neutralizing the surface charge density (Marschner and Kalbitz, 2003) mainly in hydrophilic fractions (Bingham and Cotrufo, 2016) which favor the accumulating throughout the soil profile.

The soil solute dynamics in Cerrado region are highly affected by precipitation seasonality and position within the watershed (e.g., distance from streams). The long period of dry season (April to September) accumulate N in the atmosphere that precipitate in the spring as relatively solute concentrate rainfall carrying high N content (Ciglasch et al., 2004; Markewitz et al., 2006). Once in the soil, the available water and nutrients trigger the SOM mineralization that is intensified by crop residues that remained covering the soil surface (Lilienfein et al., 2003). This period is known to its potential to leach nutrients and its intrinsic risk of environmental contamination, mainly in lowlands and areas surrounding gallery forests (Parron et al., 2011; Wilcke and Lilienfein, 2005). According to Lehmann et al. (2004), the N fertilizer applied under soybean and maize cropped soils of Cerrado was rapidly nitrified or immobilized and most of the N leached from the topsoil occurred during the following 30 days after application. Our sampling was proceeded in the end of the maize cycle which means

that the crop demand by nutrients had already been supplied by soil as well as the rainy season had been gone, so we suppose that the amount of inorganic N forms represent the net stocks in the end of the crop seasons (Fig. 4). Indeed, the high grain yields of soybean and maize linked to high N cycling in soil constrained N leaching in the current study. However, the accumulated nitrate at 0.2-0.6 m layer only in CRN4 revealed that applying high N rates (210 kg ha^{-1} yr^{-1}) might create favorable conditions to nitrate leaching, partially confirming our hypothesis (ii).

The SMPS and N fertilizer effects on DOC and DON are linked to the decomposition process of organic matter which were more pronounced in the 0-0.2 m soil layer (Fig. 5). With the lowest annual N inputs, DCN1 had the greater DON production in alignment to the decomposition rates of slow and passive pools (Table 4), suggesting that the double cropping under no N fertilizer promote decomposition of more stable soil organic matter to supply N demands of the growing plants. In N-limited systems, the depolymerization rates of complex compounds by microbial biomass regulates the overall N cycling in which the products (i.e. monomers, dimers) are used by plants and microorganisms at very low rates of mineralization (Chen et al., 2014; Jones et al., 2009; Schimel and Bennett, 2004). Thus, we consider that DCN1 may have a negative effect on soil N dynamics since the decomposition process could trigger the depletion of soil N along with continuous cropping at low N inputs.

3.4.3 Soil C mineralization kinetics and enzymatic activities

Up to date, we do not know similar study of soil C mineralization kinetics with Cerrado Oxisols over longer periods of incubation (500 d). Because of the long duration we could use a triphasic exponential decay model that allowed to estimate greater number of significative parameters (6) with relative minimum error and better dependency values of the parameters model (Glanville et al., 2016). Otherwise, the biphasic model would not detail the soil C mineralization kinetics by occluding the C_s into C_a and C_p.

In general, the soil C mineralization kinetics of NV was less conservative than the other treatments (Fig. 6 and Table 4). Not only the kinetics but also the accumulated and relative CO_2-C emissions in the end of incubation were high (Table 3). Actually, considering that the topsoil texture from the experiment and NV are equal we could expect a C stabilization directly depending on the rates of annually C added as biomass up to a saturation limit related to the land use or management practice adopted (Souza

et al., 2017; Stewart et al., 2007). However, the annual C inputs of 4.3 Mg ha^{-1} estimated for Cerrado vegetation (Corbeels et al., 2006) is far below those estimated in our experiment whereas the C stocks of NV surpass those measured in the SMPS treatments (Fig. 3) which indicate low C saturation deficit for NV. Thus, since the steady state on soil C dynamics of NV had been reached, the protective mechanisms of soil C may be saturated and most of the annual C input as particulate organic matter is unprotected and passive to mineralization process (Stewart et al., 2008). In fact, these effects on C dynamics might be expected since 8% to 20% of SOC derive from particulate organic C in tropical forests depending on the clay content (Motavalli et al., 1994). Likewise, the SMPS were far from the soil C saturation reference (i.e., NV) but the high annual C inputs estimated on CR have been decreasing the C saturation deficit which is evident through the more conservative soil C mineralization kinetics. Still, we suppose that CR is near to a significant increase on soil C stocks related to DC, further supporting the importance of this long-term experiments.

The soil C mineralization kinetics showed a pronounced effect of N fertilizer by raising the decay constants of all decomposition phases which indicate a boost in nutrient cycling. This effect was found by Neff et al. (2002) to accelerate the decay rate of light fractions (i.e. particulate organic matter) at decadal-aged but stabilize C compounds in mineral-associated fractions at multidecadal- to century-aged. The authors also found significantly high soil C inputs on N added plots without affecting the soil C stocks that highlight the limitation of applying a single-phase model approach (whole soil) to elucidate C changes responses by experienced factors. Similar results were obtained in a range of Cerrado agrosystems in which increasing fertilization levels stimulated the decomposition of native vegetation derived-C (de Sant-Anna et al., 2017). Another line of evidence that converge to the boosted nutrient cycling induced by N fertilization was already discussed, like the increase of C and N inputs on soil not followed by C and N stocks.

The results from the long-term mineralization assay elucidated the importance of temporal evaluations in detecting the effect of factors involved in the decomposition process. Even under nine years experiencing different agrosystems (DC and CR) and N fertilizer levels, only few significant differences were found on topsoil parameters (i.e., DON) while remarkable differences were showed as function of C mineralization kinetics over time, like DC to CR and inferior (N1 and N2) to superior N levels (N3 and N4). Despite that, there was no difference between the C_a (active phase) at all which

denotes the matter of temporal evaluation for long-term incubation (data not shown). We also expected some differences in BGC and BGN considering the results accessing crop rotation and N fertilizer effects on enzymatic activities found on literature (Bonini Pires et al., 2020; Ekenler and Tabatabai, 2002; Tiemann et al., 2015). However, the dissolved organic forms of C and N content were negatively correlated to BGC (r = -0.274; p = 0.087) and BGN (r = -0.360; p = 0.023), respectively, indicating that higher content/availability of dissolved forms inhibited the enzymatic activities. This statement suggest that DOC and DON are enriched by labile compounds as proposed by Ros et al. (2009).

3.5 Conclusions

Not surprisingly, the land use change effect impacted more consistently the soil C and N stocks as compared to changes promoted by agricultural management practices (crop succession/rotation or N fertilization) within the soybean and maize production system. Nine years of agrosystem intensification through adoption of crop rotation (legumes and grasses) or maximization of N-fertilizer usage did not modify soil C and N stocks as compared to the currently soybean-maize succession with moderate levels of N. Conversely, clear improvements on grain yield and C and N inputs were detected in maize crop following crop rotation and higher N level.

Native vegetation presented higher stocks of dissolved forms of C and N on soil as compared to soybean and maize production systems. The moderate N rates evaluated in this study, aligned to a rapid growth and N uptake by maize, indicated a relative low potential for nitrate leaching. In opposite, combined adoption of crop rotation with high N rates increased the potential of nitrate enrichment in the subsoil. A 500-d incubation assay revealed an increased soil C mineralization in the soil from native vegetation as compared to the soybean and maize production systems while crop rotation system presented a more conservative soil C mineralization kinetics. However, increasing N fertilization levels promoted a decreasing on active and slow pools and stimulated the decomposition constant of all pools that may indicates a boost on nutrient cycling.

Technically, soybean-maize succession allows the harvest of two cash crops every year whereas crop diversification including cover- and cash crops along cycles rotation may decreases the land intensification for profitability and this is a challenge for crop rotation adoption in large-scale by farmers. Even so, soybean-maize

succession still a feasible grain production system in Cerrado region while maintaining soil C and N stocks and constraining nitrate leaching losses.

References

Alexandratos, N., Bruinsma, J., 2012. World Agriculture Towards 2030/2050: the 2012 revision. https://doi.org/10.1002/jso.2930300113

Atkinson, R.J., 1967. Adsorption of potential-determining ions at the ferric oxide-aqueous electrolyte interface. J. Phys. Chem. 71, 550–558. https://doi.org/10.1021/j100862a014

Batlle-Bayer, L., Batjes, N.H., Bindraban, P.S., 2010. Changes in organic carbon stocks upon land use conversion in the Brazilian Cerrado: A review. Agric. Ecosyst. Environ. 137, 47–58. https://doi.org/10.1016/j.agee.2010.02.003

Bayer, C., Martin-Neto, L., Mielniczuk, J., Pavinato, A., Dieckow, J., 2006. Carbon sequestration in two Brazilian Cerrado soils under no-till. Soil Tillage Res. 86, 237–245. https://doi.org/10.1016/j.still.2005.02.023

Bingham, A.H., Cotrufo, M.F., 2016. Organic nitrogen storage in mineral soil: Implications for policy and management. Sci. Total Environ. 551–552, 116–126. https://doi.org/10.1016/j.scitotenv.2016.02.020

Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A., Hergert, G.W., 2015. Cover crops and ecosystem services: Insights from studies in temperate soils. Agron. J. 107, 2449–2474. https://doi.org/10.2134/agronj15.0086

Bonini Pires, C.A., Amado, T.J.C., Reimche, G., Schwalbert, R., Sarto, M.V.M., Nicoloso, R.S., Fiorin, J.E., Rice, C.W., 2020. Diversified crop rotation with no-till changes microbial distribution with depth and enhances activity in a subtropical Oxisol. Eur. J. Soil Sci. 1–15. https://doi.org/10.1111/ejss.12981

Brando, P.M., Coe, M.T., DeFries, R., Azevedo, A.A., 2013. Ecology, economy and management of an agroindustrial frontier landscape in the southeast Amazon. Philos. Trans. R. Soc. B Biol. Sci. 368. https://doi.org/10.1098/rstb.2012.0152

Bustamante, M.M.C., Martinelli, L.A., Silva, D.A., Camargo, P.B., Klink, C.A., Domingues, T.F., Santos, R. V., 2004. 15N natural abundance in woody plants and soils of Central brazilian savannas (cerrado). Ecol. Appl. 14, 200–213. https://doi.org/10.1890/01-6013

Bustamante, M.M.C., Medina, E., Asner, G.P., Nardoto, G.B., Garcia-Montiel, D.C., 2006. Nitrogen cycling in tropical and temperate savannas. Biogeochemistry 79, 209–237. https://doi.org/10.1007/s10533-006-9006-x

Cabrera, M.L., Beare, M.H., 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci. Soc. Am. J. 57, 1007. https://doi.org/10.2136/sssaj1993.03615995005700040021x

Calegari, A., Hargrove, W.L., Rheinheimer, D.D.S., Ralisch, R., Tessier, D., De Tourdonnet, S., Guimarães, M.D.F., 2008. Impact of long-term no-tillage and cropping system management on soil organic carbon in an oxisol: A model for sustainability. Agron. J. 100, 1013–1019. https://doi.org/10.2134/agronj2007.0121

Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E., Kuzyakov, Y., 2014. Soil C and N availability determine the priming effect: Microbial N mining and stoichiometric decomposition theories. Glob. Chang. Biol. 20, 2356–2367. https://doi.org/10.1111/gcb.12475

Chivenge, P., Vanlauwe, B., Gentile, R., Six, J., 2011. Organic resource quality influences short-term aggregate dynamics and soil organic carbon and nitrogen accumulation. Soil Biol. Biochem. 43, 657–666. https://doi.org/10.1016/j.soilbio.2010.12.002

Ciglasch, H., Lilienfein, J., Kaiser, K., Wilcke, W., 2004. Dissolved organic matter under native Cerrado and Pinus caribaea plantations in the Brazilian savanna. Biogeochemistry 67, 157–182. https://doi.org/10.1023/B:BIOG.0000015281.74705.f8

Cleveland, C.C., Liptzin, D., 2007. C:N:P stoichiometry in soil: Is there a “Redfield ratio” for the microbial biomass? Biogeochemistry 85, 235–252. https://doi.org/10.1007/s10533-007-9132-0

Coelho, M.J.A., 2019. Legacy phosphorus accumulation under long-term soil and fertilizer management in crop production Piracicaba 2019.

Corbeels, M., Scopel, E., Cardoso, A., Bernoux, M., Douzet, J.M., Neto, M.S., 2006. Soil carbon storage potential of direct seeding mulch-based cropping systems in the Cerrados of Brazil. Glob. Chang. Biol. 12, 1773–1787. https://doi.org/10.1111/j.1365-2486.2006.01233.x

Crusciol, C.A.C., Nascente, A.S., Borghi, E., Soratto, R.P., Martins, P.O., 2015. Improving soil fertility and crop yield in a tropical region with palisadegrass cover crops. Agron. J. 107, 2271–2280. https://doi.org/10.2134/agronj14.0603

de Brito, G.S., Bautista, S., López-Poma, R., Pivello, V.R., 2019. Labile soil organic carbon loss in response to land conversion in the Brazilian woodland savanna (cerradão). Biogeochemistry 144, 31–46. https://doi.org/10.1007/s10533-019-00570-9

de Freitas, P.L., Landers, J.N., 2014. The transformation of agriculture in Brazil through development and adoption of zero tillage conservation agriculture. Int. Soil Water Conserv. Res. 2, 35–46. https://doi.org/10.1016/S2095-6339(15)30012-5

de Sant-Anna, S.A.C., Jantalia, C.P., Sá, J.M., Vilela, L., Marchão, R.L., Alves, B.J.R., Urquiaga, S., Boddey, R.M., 2017. Changes in soil organic carbon during 22 years of pastures, cropping or integrated crop/livestock systems in the Brazilian Cerrado. Nutr. Cycl. Agroecosystems 108, 101–120. https://doi.org/10.1007/s10705-016-9812-z

Dieckow, J., Bayer, C., Conceição, P.C., Zanatta, J.A., Martin-Neto, L., Milori, D.B.M., Salton, J.C., MacEdo, M.M., Mielniczuk, J., Hernani, L.C., 2009. Land use, tillage, texture and organic matter stock and composition in tropical and subtropical Brazilian soils. Eur. J. Soil Sci. 60, 240–249. https://doi.org/10.1111/j.1365-2389.2008.01101.x

Dilly, O., 2006. Soil Microbial Activity, in: Microbiological Methods for Assessing Soil Quality. pp. 114–182. https://doi.org/10.1079/9780851990989.0000

Don, A., Schumacher, J., Freibauer, A., 2011. Impact of tropical land-use change on soil organic carbon stocks - a meta-analysis. Glob. Chang. Biol. 17, 1658–1670. https://doi.org/10.1111/j.1365-2486.2010.02336.x

Ekenler, M., Tabatabai, M.A., 2002. β-glucosaminidase activity of soils: Effect of cropping systems and its relationship to nitrogen mineralization. Biol. Fertil. Soils 36, 367–376. https://doi.org/10.1007/s00374-002-0541-x

Ellert, B.H., Bettany, J.R., 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. Can. J. Soil Sci. 75, 529–538. https://doi.org/10.4141/cjss95-075

FAO, 2018. The State of Agricultural Commodity Markets: Agricultural Trade, Climate Change and Food Security.

Ferreira, A.O., Amado, T.J.C., Nicoloso, R.S., Sá, J.C.M., Fiorin, J.E., Hansel, D.S.S., Menefee, D., 2013. Soil carbon stratification affected by long-term tillage and cropping systems in southern Brazil. Soil Tillage Res. 133, 65–74. https://doi.org/10.1016/j.still.2013.05.011

Glanville, H.C., Hill, P.W., Schnepf, A., Oburger, E., Jones, D.L., 2016. Combined use of empirical data and mathematical modelling to better estimate the microbial turnover of isotopically labelled carbon substrates in soil. Soil Biol. Biochem. 94, 154–168. https://doi.org/10.1016/j.soilbio.2015.11.016

Goedert, W.J., 1983. Management of the Cerrado soils of Brazil : a review. J. Soil Sci. 34, 405–428.

Grabau, Z.J., Chen, S., 2016. Influence of long-term corn-soybean crop sequences on soil ecology as indicated by the nematode community. Appl. Soil Ecol. 100, 172–185. https://doi.org/10.1016/j.apsoil.2015.12.016

Haney, R.L., Franzluebbers, A.J., Porter, E.B., Hons, F.M., Zuberer, D.A., 2004. Soil carbon and nitrogen mineralization: influence of drying temperature. Soil Sci. Soc. Am. J. 68, 489–492. https://doi.org/10.2136/sssaj2004.4890

Hessen, D.O., Ågren, G.I., Anderson, T.R., Elser, J.J., De Ruiter, P.C., 2004. Carbon sequestration in ecosystems: The role of stoichiometry. Ecology 85, 1179–1192. https://doi.org/10.1890/02-0251

Hosono, A., da Rocha, C.M.C., Hongo, Y., 2016. Introduction: Development of Cerrado Agriculture, in: Development for Sustainable Agriculture: The Brazilian Cerrado. pp. 1–7. https://doi.org/10.1057/9781137431356_1

Hosono, A., Hongo, Y., 2016a. Technological Innovations That Made Cerrado Agriculture Possible, in: Development for Sustainable Agriculture: The Brazilian Cerrado. pp. 11–34. https://doi.org/10.1057/9781137431356_2

Hosono, A., Hongo, Y., 2016b. Development of Cerrado Agriculture: The Path to Becoming a Major Global Breadbasket, in: Development for Sustainable Agriculture: The Brazilian Cerrado. pp. 61–90. https://doi.org/10.1057/9781137431356_4

Hunke, P., Mueller, E.N., Schröder, B., Zeilhofer, P., 2015. The Brazilian Cerrado: Assessment of water and soil degradation in catchments under intensive agricultural use. Ecohydrology 8, 1154–1180. https://doi.org/10.1002/eco.1573

INPE, 2015. Projeto TerraClass Cerrado 2013, Mapeamento do Uso e Cobertura do Cerrado.

Islan, K.R., Weil, R.R., 1998. Microwave irradiation of soil for routine measurement of microbial biomass carbon. Biol. Fertil. Soils 408–416. https://doi.org/10.1109/RADAR.2014.7060241

Jackson, P.C., Meinzer, F.C., Bustamante, M., Goldstein, G., Franco, A., Rundel, P.W., Caldas, L., Igler, E., Causin, F., 1999. Partitioning of soil water among tree species in a Brazilian Cerrado ecosystem. Tree Physiol. 19, 717–724. https://doi.org/10.1093/treephys/19.11.717

Jones, D.L., Kielland, K., Sinclair, F.L., Dahlgren, R.A., Newsham, K.K., Farrar, J.F., Murphy, D. V., 2009. Soil organic nitrogen mineralization across a global latitudinal gradient. Global Biogeochem. Cycles 23, 1–5. https://doi.org/10.1029/2008GB003250

Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem. 38, 991–999. https://doi.org/10.1016/j.soilbio.2005.08.012

Kalbitz, K., Solinger, S., Park, J.-H., Michalzik, B., Matzner, E., 2000. Controls on the dynamics of dissolved organic matter in soils A review.pdf. Soil Sci. 165, 277–304.

Kauer, K., Tein, B., Sanchez de Cima, D., Talgre, L., Eremeev, V., Loit, E., Luik, A., 2015. Soil carbon dynamics estimation and dependence on farming system in a temperate climate. Soil Tillage Res. 154, 53–63. https://doi.org/10.1016/j.still.2015.06.010

Khan, S.A., Mulvaney, R.L., Ellsworth, T.R., Boast, C.W., 2007. The myth of nitrogen fertilization for soil carbon sequestration. J. Environ. Qual. 36, 1821–1832. https://doi.org/10.2134/jeq2007.0099

Khan, S.A., Mulvaney, R.L., Mulvaney, C.S., 1997. Accelerated diffusion methods for inorganic-nitrogen analysis of soil extracts and water. Soil Sci. Soc. Am. J. 61, 936. https://doi.org/10.1063/1.101628

Kirkby, C.A., Kirkegaard, J.A., Richardson, A.E., Wade, L.J., Blanchard, C., Batten, G., 2011. Stable soil organic matter: A comparison of C:N:P:S ratios in Australian and other world soils. Geoderma 163, 197–208. https://doi.org/10.1016/j.geoderma.2011.04.010

Lee, J., Hopmans, J.W., Rolston, D.E., Baer, S.G., Six, J., 2009. Determining soil carbon stock changes: Simple bulk density corrections fail. Agric. Ecosyst. Environ. 134, 251–256. https://doi.org/10.1016/j.agee.2009.07.006

Lehmann, J., Lehmann, J., Lilienfein, J., Rebel, K., do Carmo Lima, S., Wilcke, W., 2004. Subsoil retention of organic and inorganic nitrogen in a Brazilian savanna Oxisol. Soil Use Manag. 20, 163–172. https://doi.org/10.1079/sum2004240

Lilienfein, J., Wilcke, W., Vilela, L., Ayarza, M.A., Lima, C., Zech, W., 2003. Soil Fertility under Native Cerrado and Pasture in the Brazilian Savanna. Soil Sci. Soc. Am. J. 1195–1205. https://doi.org/10.2136/sssaj2003.1195

Luo, Z., Wang, E., Sun, O.J., 2010. Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. Agric. Ecosyst. Environ. 139, 224–231. https://doi.org/10.1016/j.agee.2010.08.006

Maltas, A., Corbeels, M., Scopel, E., Oliver, R., Douzet, J.M., Da Silva, F.A.M.E., Wery, J., 2007. Long-term effects of continuous direct seeding mulch-based cropping systems on soil nitrogen supply in the Cerrado region of Brazil. Plant Soil 298, 161–173. https://doi.org/10.1007/s11104-007-9350-1

Markewitz, D., Resende, J.C.F., Parron, L., Bustamante, M., Klink, C.A., Figueiredo, R.D.O., Davidson, E.A., 2006. Dissolved rainfall inputs and streamwater outputs in an undisturbed watershed on highly weathered soils in the Brazilian cerrado. Hydrol. Process. 2639, 2615–2639. https://doi.org/10.1002/hyp

Marschner, B., Kalbitz, K., 2003. Controls of bioavailability and biodegradability of dissolved organic matter in soils. Geoderma 113, 211–235. https://doi.org/10.1016/S0016-7061(02)00362-2

McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? a meta-analysis. Ecol. Appl. 24, 560–570. https://doi.org/10.1890/13-0616.1

Mekaru, T., Uehara, G., 1972. Anion Adsorption in Ferruginous Tropical Soils. Soil Sci. Soc. Am. Proc. 36, 296–300.

Michalzik, B., Kalbitz, K., Park, J.H., Solinger, S., Matzner, E., 2001. Fluxes and concentrations of dissolved organic carbon and nitrogen - A synthesis for temperate forests. Biogeochemistry 52, 173–205. https://doi.org/10.1023/A:1006441620810

Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide Biol. Chem. 5, 62–71. https://doi.org/10.1006/niox.2000.0319

Motavalli, P.P., Palm, C.A., Parton, W.J., Elliott, E.T., Frey, S.D., 1994. Comparison of laboratory and modeling simulation methods for estimating soil carbon pools in tropical forest soils. Soil Biol. Biochem. 26, 935–944. https://doi.org/10.1016/0038-0717(94)90106-6

Mulvaney, R.L., 1996. Nitrogen - Inorganic Forms, in: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H. (Eds.), Methods of Soil Analysis Part 3 - Chemical Methods. Soil Science Society of America, American Society of Agronomy, Madison, pp. 1123–1184. https://doi.org/10.2136/sssabookser5.3.c38

Mulvaney, R.L., Khan, S.A., Ellsworth, T.R., 2009. Synthetic nitrogen fertilizers deplete soil nitrogen: a global dilemma for sustainable cereal production. J. Environ. Qual. 38, 2295–2314. https://doi.org/10.2134/jeq2008.0527

Murty, D., Kirschbaum, M.U.F., Mcmurtrie, R.E., Mcgilvray, H., 2002. Does conversion of forest to agricultural land change soil carbon and nitrogen? A review of the literature. Glob. Chang. Biol. 8, 105–123. https://doi.org/10.1046/j.1354-1013.2001.00459.x

Neff, J.C., Townsend, A.R., Gleixner, G., Lehman, S.J., Turnbull, J., Bowman, W.D., 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419, 915–917. https://doi.org/10.1038/nature01136

Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter, in: Methods of Soil Analysis: Part 3— Chemical Methods. pp. 961–1010.

Neto, M.S., Scopel, E., Corbeels, M., Cardoso, A.N., Douzet, J.M., Feller, C., Piccolo, M. de C., Cerri, C.C., Bernoux, M., 2010. Soil carbon stocks under no-tillage mulch-based cropping systems in the Brazilian Cerrado: An on-farm synchronic assessment. Soil Tillage Res. 110, 187–195. https://doi.org/10.1016/j.still.2010.07.010

Oliveira-Filho, E.C., Brito, D.Q., Dias, Z.M.B., Guarieiro, M.S., Carvalho, E.L., Fascineli, M.L., Niva, C.C., Grisolia, C.K., 2018. Effects of ashes from a Brazilian savanna wildfire on water, soil and biota: An ecotoxicological approach. Sci. Total Environ. 618, 101–111. https://doi.org/10.1016/j.scitotenv.2017.11.051

Owens, B., 2013. Long-term research: Slow science. Nature 495, 300–303. https://doi.org/10.1038/495300a

Palm, C., Blanco-Canqui, H., DeClerck, F., Gatere, L., Grace, P., 2014. Conservation agriculture and ecosystem services: An overview. Agric. Ecosyst. Environ. 187, 87–105. https://doi.org/10.1016/j.agee.2013.10.010

Parham, J.A., Deng, S.P., 2000. Detection, quantification and characterization of B-glucosaminidase activity in soil. Soil Biol. Biochem. 32, 1183–1190.

Parks, G.A., De Bruyn, P.L., 1962. The zero point of charge of oxides. J. Phys. Chem. 66, 967–973. https://doi.org/10.1021/j100812a002

Parron, L.M., Bustamante, M.M. da C., Markewitz, D., 2011. Fluxes of nitrogen and phosphorus in a gallery forest in the Cerrado of central Brazil. Biogeochemistry 89–104. https://doi.org/10.1007/s10533-010-9537-z

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2018. nlme: Linear and Nonlinear Mixed Effects Models.

Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops - A meta-analysis. Agric. Ecosyst. Environ. 200, 33–41. https://doi.org/10.1016/j.agee.2014.10.024

Quesada, C.A., Hodnett, M.G., Breyer, L.M., Santos, A.J.B., Andrade, S., Miranda, H.S., Miranda, A.C., Lloyd, J., 2008. Seasonal variations in soil water in two woodland

savannas of central Brazil with different fire history. Tree Physiol. 28, 405–415. https://doi.org/10.1093/treephys/28.3.405

R Core Team, 2019. R: A language and environment for statistical computing.

Rausch, L.L., Gibbs, H.K., Schelly, I., Jr, A.B., Morton, D.C., Filho, A.C., Strassburg, B., Walker, N., Noojipady, P., Barreto, P., Meyer, D., 2019. Soy expansion in Brazil ' s Cerrado. Conserv. Lett. 1–10. https://doi.org/10.1111/conl.12671

Riggs, C.E., Hobbie, S.E., Bach, E.M., Hofmockel, K.S., Kazanski, C.E., 2015. Nitrogen addition changes grassland soil organic matter decomposition. Biogeochemistry 125, 203–219. https://doi.org/10.1007/s10533-015-0123-2

Ritz, C., Streibig, J.C., 2009. Grouped Data, in: Nonlinear Regression with R. https://doi.org/10.1002/0471667196.ess0909

Ros, G.H., Hoffland, E., van Kessel, C., Temminghoff, E.J.M., 2009. Extractable and dissolved soil organic nitrogen - A quantitative assessment. Soil Biol. Biochem. 41, 1029–1039. https://doi.org/10.1016/j.soilbio.2009.01.011

Sano, E.E., Rosa, R., Brito, J.L.S., Ferreira, L.G., 2010. Land cover mapping of the tropical savanna region in Brazil. Environ. Monit. Assess. 166, 113–124. https://doi.org/10.1007/s10661-009-0988-4

Santos, N.Z.D., Dieckow, J., Bayer, C., Molin, R., Favaretto, N., Pauletti, V., Piva, J.T., 2011. Forages, cover crops and related shoot and root additions in no-till rotations to C sequestration in a subtropical Ferralsol. Soil Tillage Res. 111, 208–218. https://doi.org/10.1016/j.still.2010.10.006

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591–602. https://doi.org/10.1890/03-8024

Schwesig, D., Kalbitz, K., Matzner, E., 2003. Effects of aluminium on the mineralization of dissolved organic carbon derived from forest floors. Eur. J. Soil Sci. 54, 311–322. https://doi.org/10.1046/j.1365-2389.2003.00523.x

Scopel, E., Triomphe, B., Affholder, F., Da Silva, F.A.M.E., Corbeels, M., Xavier, J.H.V., Lahmar, R., Recous, S., Bernoux, M., Blanchart, E., De Carvalho Mendes, I., De Tourdonnet, S., 2013. Conservation agriculture cropping systems in temperate and tropical conditions, performances and impacts. A review. Agron. Sustain. Dev. 33, 113–130. https://doi.org/10.1007/s13593-012-0106-9

Sinsabaugh, R.L., Follstad Shah, J.J., 2012. Ecoenzymatic Stoichiometry and Ecological Theory. Annu. Rev. Ecol. Evol. Syst. 43, 313–343. https://doi.org/10.1146/annurev-ecolsys-071112-124414

Six, J., Conant, R.T., Paul, E. a, Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturatin of soils. Plant Soil 241, 155–176. https://doi.org/10.1023/A:1016125726789

Soil Survey Staff, 2014. Keys to soil taxonomy, Soil Conservation Service.

Souza, I.F., Almeida, L.F.J., Jesus, G.L., Kleber, M., Silva, I.R., 2017. The mechanisms of organic carbon protection and dynamics of C-saturation in Oxisols vary with particle-size distribution. Eur. J. Soil Sci. 68, 726–739. https://doi.org/10.1111/ejss.12463

Spehar, C.R., 2006. Conquering the Brazilian Savannah and consolidation of agriculture, in: Ciência, Agricultura e Sociedade. pp. 195–226.

Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2007. Soil carbon saturation: Concept, evidence and evaluation. Biogeochemistry 86, 19–31. https://doi.org/10.1007/s10533-007-9140-0

Stewart, C.E., Plante, A.F., Paustian, K., Conant, R.T., Six, J., 2008. Soil carbon saturation: linking concept and measurable carbon pools. Soil Sci. Soc. Am. J. 72, 379–392. https://doi.org/10.2136/sssaj2007.0104

Strassburg, B.B.N., Latawiec, A.E., Barioni, L.G., Nobre, C.A., da Silva, V.P., Valentim, J.F., Vianna, M., Assad, E.D., 2014. When enough should be enough: Improving the use of current agricultural lands could meet production demands and spare natural habitats in Brazil. Glob. Environ. Chang. 28, 84–97. https://doi.org/10.1016/j.gloenvcha.2014.06.001

Tabatabai, M.A., 1994. Soil Enzymes, in: Methods of Soil Analysis Part 2 - Chemical and Microbiological Properties. pp. 775–833. https://doi.org/10.1007/978-1-61779-558-9

Tiemann, L.K., Grandy, A.S., Atkinson, E.E., Marin-Spiotta, E., Mcdaniel, M.D., 2015. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. Ecol. Lett. 18, 761–771. https://doi.org/10.1111/ele.12453

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature 418, 671–677. https://doi.org/10.1038/nature01014

West, T.O., Post, W.M., 2002. Soil Organic Carbon Sequestration Rates by Tillage and Crop Rotation. Soil Sci. Soc. Am. J. 66, 1930–1946. https://doi.org/10.2136/sssaj2002.1930

Wilcke, W., Lilienfein, J., 2005. Nutrient Leaching in Oxisols Under Native and Managed Vegetation in Brazil. Soil Sci. Soc. Am. J. 69, 1152–1161. https://doi.org/10.2136/sssaj2004.0350

Wittwer, R.A., Dorn, B., Jossi, W., van der Heijden, M.G.A., 2017. Cover crops support ecological intensification of arable cropping systems. Sci. Rep. 7, 41911. https://doi.org/10.1038/srep41911

Wollum, A.G., 1994. Soil Sampling for Microbiological Analysis, in: Methods of Soil Analysis, Part 2: Microbiological and Biochemical Properties. pp. 1–14. https://doi.org/10.2136/sssabookser5.2.c1

Yopps, J.A., Fuerstenau, D.W., 1964. The zero point of charge of α-alumina. J. Colloid Sci. 19, 61–71.

Zegada-Lizarazu, W., Monti, A., 2011. Energy crops in rotation. A review. Biomass and Bioenergy 35, 12–25. https://doi.org/10.1016/j.biombioe.2010.08.001

Zibilske, L.M., 1994. Carbon mineralization, in: Methods of Soil Analysis, Part 2: Microbiological and Biochemical Properties. pp. 835–863.

Zinn, Y.L., Marrenjo, G.J., Silva, C.A., 2018. Soil C:N ratios are unresponsive to land use change in Brazil: A comparative analysis. Agric. Ecosyst. Environ. 255, 62–72. https://doi.org/10.1016/j.agee.2017.12.019

4 ELUCIDATING THE POTENTIAL OF SUBSOILS FOR ENHANCED CARBON STORAGE IN OXISOL

Abstract

Enhancing carbon (C) storage in subsoil has been attempting to mitigate the anthropogenic C emissions. However, we do not fully understand the factors controlling C cycling towards accumulation and preservation in the deep horizon of tropical soils. The aim of this study was to access the C decomposition kinetics of top- and subsoils of Oxisols by applying discrete C substrates, promoting the priming effect, and balancing the nutrient availability under different agroecosystems in the two most important grain producing regions in Brazil (i.e., Mato Grosso and Paraná). To elucidate our purpose, two sets of independent assays were performed addressing the soil microbial community on using: i) ^{14}C-labelled D-glucose, D-glucosamine hydrochloride and cellulose, and; ii) ^{14}C-labelled D-glucose reapplication (priming) upon nutrient availability. The decomposition kinetics of the ^{14}C-labelled substrates were estimated into exponential decay models through measurements of $^{14}CO_2$ evolution from soil and the carbon use efficiency (CUE) calculated as proxy to relate the attached factors. Overall, the decomposition patterns varied mostly within layers, substrates and priming than site, agroecosystems, and nutrient availability. On average, higher CUE were found under regional standard agrosystems, subsoils samples and cellulose application as response of soil microbial community in which Mato Grosso site had the most potential for C stabilization. Priming demonstrated that the initial lag-phase on decomposition kinetics of subsoils samples were probably related to dormant microorganisms instead of minor microbial biomass and low nutrient availability in Oxisols. Furthermore, the substrate reapplication was a significant technique allowing to decouple the $^{14}CO_2$ measurement from the first and the second applications of ^{14}C-labelled glucose and showing a decrease on CUE following glucose reapplication (priming effect) while an increase on CUE accounting the successive pulses of ^{14}C to the soil.

Keywords: Carbon use efficiency; Microbial community; Priming effect; Nutrient stoichiometric; Labelled substrates.; Exponential decay.

4.1 Introduction

Soils represent a major store of global carbon (3000 Gt C), far exceeding the amount held in vegetation (560 Gt) or the atmosphere (830 Gt CO_2-C) (Stockmann et al., 2015). This reservoir of organic C, however, is highly susceptible to being destabilized and lost, ultimately leading to a decline in soil structure, loss of biodiversity and nutrient, as well as the release of greenhouse gases to the atmosphere. Recent estimates suggest that 133 Gt of soil C has already been released to the atmosphere due to land use change (LUC) over the last 12,000 yr and this was primarily associated to the adoption of agriculture (Sanderman et al., 2017). Such C losses have not been

recovered even after decades of adequate management and fertilization conditions (Buyanovsky and Wagner, 1998; Fabrizzi et al., 2009; Khan et al., 2007; Mulvaney et al., 2009; Paul et al., 1996; Sisti et al., 2004).

If we are to achieve food security and protect the environment, it is paramount that current rates of soil C loss be abated, and where possible, reversed. This is the central tenet of the '4 per mille Soils for Food Security and Climate' initiative, which aspires to increase global soil organic matter stocks by 0.4% per year (0.16% C yr^{-1}) to compensate for the global emissions of GHGs by anthropogenic sources (Minasny et al., 2017). This has proved highly controversial within the scientific community, with many suggesting that the target is ill-conceived and unachievable, especially in the long-term (Baveye et al., 2018; de Vries, 2018; White et al., 2018). The reasons given for this include: (i) a lack of appreciation for the C saturation point of soils; (ii) overinflated estimates of potential rates of C accrual; (iii) no consideration of priming and loss of old soil C; (iv) no accounting for nutrient stoichiometry in soil organic matter (SOM) (i.e., N and P); and (v) potentially negative impacts of land use change on food security.

On highly degraded soils with low organic matter contents (<1.5% SOM; topsoil <18 t C ha^{-1}) a '4 per mille' target may be achievable (e.g., sequestration of 0.07 t C ha^{-1} y^{-1}), but not in soil of well-stablished agrosystems. In some long-term experiments, increasing C addition in the agrosystems did not reflect increments in soil C stocks (Buyanovsky and Wagner, 1998; Chung et al., 2010; Gulde et al., 2008; Stewart et al., 2007), which claims for the reasons pointed out above and weakens the benefits of the '4 per mille' initiative. In this context, it is necessary to rebuild soil organic C (SOC) where is possible (e.g., undermanaged cropping systems, degraded soils), but also preserve SOC in agrosystems where its loss appears inevitable under current management (e.g., intensive fertilized soils).

To date, most effort on SOC stocks and dynamics have focused only on topsoils (i.e., 0.3 m). In tropical regions this zone only accounts for ~34% of SOC; the remaining lies below this layer (i.e. 0.3-1.0 m) (Batjes, 1996). As a vast amount of work has already been undertaken on improving the SOC on topsoils (Abdalla et al., 2018; Guo and Gifford, 2002), evidence from numerous critical reviews suggests that subsoil is the layer in which most fundamental research is needed (Batjes, 1996; Kautz et al., 2013; Minasny et al., 2017). Further, in Brazil, whilst the impacts of management practices to increase SOC stocks on topsoils have been extensively studied (e.g. LUC,

reduced tillage, organic residues additions, fertilizers, etc.), few studies have quantified the benefits of exploring the subsoil layer (Amado et al., 2006; Batlle-Bayer et al., 2010; Boddey et al., 2010; Calegari et al., 2008; Corbeels et al., 2006; Fabrizzi et al., 2009).

Research into SOC has undergone a paradigm shift away from the concept of the recalcitrance of 'humic macromolecules' to most SOC being labile but protected through chemical, physical and biochemical stabilization mechanisms (Six et al., 2002). Devising effective management strategies to rebuild SOM requires a broad mechanistic understanding of the factors that control the C input/output to/from the system over both space and time (Kuzyakov and Blagodatskaya, 2015). Key mechanisms include inputs of new-added substrates and their transformation towards building SOM as majorly driven by microbial decomposition processes (Blagodatsky et al., 2010; Kuzyakov, 2006). Whenever accessible to microorganisms (Darrouzet-Nardi and Weintraub, 2014), the substrates are either biochemically modified by exoenzymes attached (i.e. cellulose/chitin depolymerization) (Schimel and Bennett, 2004) or readily used in metabolic pathways into microbial biomass (i.e. sugars, amino sugars, amino acids) (Hill et al., 2008; Roberts and Jones, 2012). Such microbial metabolism has been extensively studied by isotopic techniques that allow to estimate the fate of ^{14}C-labelled substrates applied in soils by simply modelling the $^{14}CO_2$ evolution released from microbial respiration. The model split the substrate-derived ^{14}C in the soil between discrete pools associated to a decreasing degree of C lability that follows an independent decay kinetic each. Thereby, once into microbial biomass, C is primarily either immobilized as structural components for growth (pool coupled to anabolic processes) or mineralized as CO_2 for cell maintenance (pool coupled to catabolic processes) (Glanville et al., 2016). From that, the understanding of the factors controlling the proportion of C use by microbial biomass towards building microbial-derived SOC by immobilization, mainly through increasing molecular size/complexity substrates added to the soil (Qiao et al., 2019) and nutrient enrichment for proper stoichiometric balance (Hessen et al., 2004), have been recognized as dependent strategies for increasing carbon use efficiency (CUE) by soil microbial biomass.

Cellulose and chitin are the most renewable abundant biopolymer in nature formed basically by long-chain of primary D-glucose and N-acetylglucosamine units respectively. In the soil-plant interface, cellulose comprises the major structural component of cell walls in plants while chitin is most found as a constituent of fungal cell walls (Kögel-Knabner, 2002). Both structural biopolymers are important sources of

C and N inputted in soils driven through depolymerization by microbial extracellular enzymes (Schimel and Bennett, 2004). The depolymerization of cellulose and chitin by enzymatic catalysis (i.e. cellulases and chitinases) produces oligomers and monomers that may be readily taken up by microbial biomass or plant roots (Hill et al., 2008; Roberts and Jones, 2012). In croplands and ecosystems, glucose is the dominant sugar (Gunina and Kuzyakov, 2015) and glucosamine contributes between 47-68% to amino sugars in topsoil (Joergensen, 2018). The importance of such substrates is thought to be even more relevant in high-weathered soils under (sub)tropical climate considering the nutrient constraining in acid soils and the rate of substrate decomposition in high temperatures (Achat et al., 2016).

The nutrient constraining (e.g. C, N, P, S) in soils has been known as an important limiting factor for plants and microorganisms lifecycle (Elser et al., 2007; Hobbie and Hobbie, 2013; Vitousek and Howarth, 1991). This limitation is most expressed in (sub)tropical regions where nutrient constraining co-exist (i.e. N, P) in high-weathered soils (e.g. Oxisol) (Harpole et al., 2011). Considering as a strategy for SOC enhancing, the stoichiometric balance of nutrient has reached controversial results: while nutrient enrichment may favor SOM formation by anabolic processes of microbial decomposition from assembly of microbial-derived substrates, nutrient exhaustion may favor SOM mining (Sinsabaugh et al., 2013; Sinsabaugh and Follstad Shah, 2012). Even so, enhancing SOC under nutrient enrichment depends on stabilization mechanisms interacting with the new formed microbial-derived substrates, like aggregation (physical protection), organo-mineral associations (chemical protection) or humification (biochemical protection) (Kirkby et al., 2016, 2014, 2013). However, nutrient addition (e.g., C, N, P, S) may promote priming decomposition of SOM, in which the successive nutrient inputs on soil acts as a trigger for boosting the SOM mineralization.

We currently do not fully understand what controls C accumulation and preservation at depth. Without a dynamic understanding of controls on C cycling throughout the entire soil profile, initiatives aimed at offsetting anthropogenic CO_2 emissions through enhanced soil C accumulation, such as ‘4 per mille’, risk being ineffective at best, or counterproductive at worst. Our overarching hypothesis is that subsoils represent a potential layer for enhanced C storage within Oxisols due to their higher water content, reduced O_2 status, lower microbial activity, and abundance of unsaturated mineral sorption surfaces. This study seeks to improve our understanding

of the C decomposition dynamics in top- and subsoil to promote C storage and greater sustainability in the two most important grain producing regions in Brazil (i.e., Mato Grosso and Paraná) under different agrosystems. In alignment, the aims of this study were: i) to assess the dynamics of the soil microbial community on using C sources of increasing molecular size/complexity adding ^{14}C-labelled substrates; ii) to evaluate the response of the soil microbial community to ^{14}C-labelled glucose reapplication upon nutrient availability on $^{14}CO_2$ priming release.

4.2 Material and methods

4.2.1 Sites description and sampling

Two long-term field experiments were selected in the two most important grain producing regions in Brazil for this study: one located in Itiquira-MT (IT: 17°09′18“S, 54°45′15“W, 490 m) and the other in Ponta Grossa-PR (PG: 25°00′46“S, 49°19′28“W, 885 m). The climate at IT is humid tropical savannah, with a dry winter and heavy rains during the summer, and at PG is humid subtropical, with regular rainfall and without dry season (Alvares et al., 2013b). The soils were classified as Typic Haplustox and Rhodic Kandiudox at IT and PG sites respectively (Soil Survey Staff, 2014). The experiments have been testing since 2009 (IT) and 2010 (PG) agrosystems involving grain production (i.e., maize and soybean) relating the regional standard agrosystem (SA) to the intensified agrosystem (IA) aimed to increase agricultural yield and mitigate adverse environmental effects. The agrosystems involved on this study were: double cropping on continually succession of soybean followed by maize at IT (SA_{IT}); crop rotation involving soybean, maize, *Crotalaria* spp. and *Brachiaria* spp. in a three-year rotation cycle at IT (IA_{IT}); soybean, maize, wheat and black oat (*Avena strigosa* Schreb.) in a two-year rotation cycle at PG (SA_{PG}), and; soybean, maize, wheat, black oat and vetch (*Vigna sativa* L.) in a four-year rotation cycle at PG (IA_{PG}). The experiments were installed under a randomized complete block design with four replicates. Further details regarding clime descriptions, land use, experimental setup and agrosystems management are available in Section 3.2 and Mira (2020).

In autumn 2018, after nine year in IT and eight year in PG, soil sampling was performed in both experiment on each plot at the 0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6, 0.6-0.8 and 0.8-1.0 m layer using an auger. Similarly, a representative native vegetation from Cerrado (wooded savannah) and Atlantic forest ecosystems (ES) respectively in IT and PG were chosen as reference areas for soil sampling. The ES areas were

chosen ~4 km apart from their field experiments to reduce the climate influence (e.g., rainfall, temperature) and to ensure an equivalent soil physicochemical characterization (e.g., clay content, CEC; Table 1). Into ES areas, four pseudo-replications (~600 m^2-size) were delimited ~50 m apart each other and randomly sampled at the same soil layers described previously. The individual samples (n = 6 per layer per plot) were then combined and mixed to obtain a composite soil sample for each layer and plot. The samples were kept on ice until transfer to the laboratory to be chilled at 4 °C for biochemical analysis and further oven-dried at 40 °C and ground to pass through 2.0 mm-sieve for chemical analysis also available in Section 3.2 and Mira (2020). In this study, only samples from 0-0.1 m (topsoil) and 0.8-1.0 m (subsoil) layers were selected to be evaluated. With that samples we performed two sets of experimental approaches: firstly, we assessed dynamics of the soil microbial community to use C sources of increasing molecular size/complexity adding ^{14}C-labelled substrates, and; second, we evaluated the response of the soil microbial community to ^{14}C-labelled glucose reapplication upon nutrient availability on $^{14}CO_2$ priming release.

Table 1. Physical and chemical soil characterization from top- and subsoil layers of the field experiment and at a representative undisturbed native vegetation in both sites

Depth	pH	OC	P	Ca	Mg	K	Al	H+Al	CEC	BS	m	Sand	Silt	Clay
m	H_2O	g kg^{-3}	mg kg^{-3}	mmol$_c$ kg^{-3}						%		g kg^{-1}		
IT_{FE}														
0-0.1	5.7	22.6	14	32	14	1.4	4	75	122.4	39	8	294	72	634
0.8-1.0	5.9	9.3	*2	9	3	0.7	*1	24	36.7	35	1	241	39	720
IT_{NV}														
0-0.1	4.4	31.3	3	6	1	1.1	32	114	122.1	7	80	298	66	636
0.8-1.0	4.4	9.3	*2	1	*1	*0.5	*1	42	43.2	4	1	249	76	675
PG_{FE}														
0-0.1	5.3	24.3	54	23	11	3.6	6	52	89.6	42	14	660	16	324
0.8-1.0	5.9	7.8	*2	8	4	1	2	25	38	34	13	533	20	447
PG_{NV}														
0-0.1	4.3	33.1	3	5	2	1.1	27	98	106.1	8	77	653	22	325
0.8-1.0	4.1	7.2	2	1	*1	*0.5	*1	27	29.5	8	1	540	17	443

IT_{FE}: Itiquira`s field experiment; $PG_{FE}$: Ponta Grossa`s field experiment; IT_{NV}: Itiquira`s native vegetation; $PG_{FE}$: Ponta Grossa`s native vegetation; OC: organic carbon; H+Al: potential acidity; CEC: cation exchange capacity at pH 7.0; BS: bases saturation; m: Al saturation; *: values below detection limit.

4.2.2 Soil (bio)chemical analyses

To provide a soil background surrounding the substrates decomposition kinetics, soil (bio)chemical analyses of microbial biomass, dissolved and total C and N were performed. For microbial biomass C (MBC) and N (MBN) analysis, two replicates of each soil sample were submitted to pre-incubation to restore their microbial activity (Haney et al., 2004). Therefore, 5 g of dried soil were weighted into 50 mL-centrifuge tubes, rewetted to bring their moisture to 50% of water-holding capacity (WHC) and gently hand mixed for water redistribution. Following, the soil samples were slightly pressured to level and standardize the soil bulk density to 1.1 g cm^{-3} and left open in the dark at 25 °C for 72 h. Immediately after pre-incubation, one replicate was extracted adding 0.5 M K_2SO_4 to the soil at a 1:5 ratio (w/v) at room temperature, shaken horizontally for 60 min at 200 rev min^{-1} and centrifuged for 10 min at 13,000 g (Jones and Willett, 2006), while the another replicate was fumigated with chloroform for 10 days (Vance et al., 1987) and then extracted as their respective non-fumigated samples.

The dissolved organic C (DOC) and total dissolved N (TDN) were determined in all extracts at high-temperature combustion method in duplicate using a CN autosampler analyser (Multi NC 2100S, AnalytikJena, Germany) and the microbial biomass was calculated as the difference between fumigated and non-fumigated extracts. Furthermore, non-fumigated extracts were determined for their dissolved inorganic N forms (DIN) through colorimetric methods into 96-well microplates and quantified in a microplate reader (PowerWave HT, BioTek™, USA). Briefly, ammonium content was determined by the salicylate–hypochlorite method followed colour reading at 667 nm (Mulvaney, 1996) while nitrate + nitrate (nitrate) content was determined by acid vanadium reduction of nitrate to nitrite and further Griess–Ilosvay reaction followed colour reading at 540 nm (Miranda et al., 2001). Dissolved organic nitrogen (DON) was calculated as the difference between TDN and DIN.

The total organic C (TOC) and N (TN) were determined in samples ground to pass through 0.149 mm-sieve by dry combustion method in duplicate using a CN autosampler analyser (CN628, LECO Corp., USA). Before combustion some drops of 4 M HCl were added to aliquots of soil samples to confirm absence of carbonates.

4.2.3 Kinetics of ^{14}C-labelled substrates decomposition

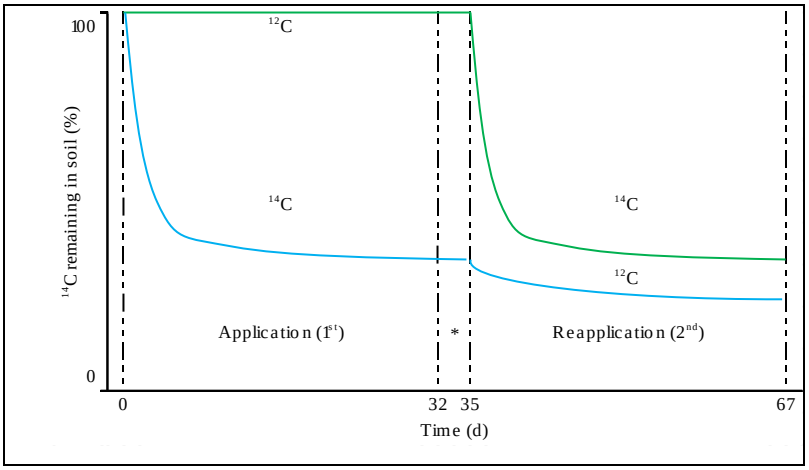
The dynamics of soil microbial community to use C sources of increasing molecular size/complexity was assessed adding ^{14}C-labelled substrates and measuring the $^{14}CO_2$ release during incubation time. The substrates involved ^{14}C-labelled D-glucose, D-glucosamine hydrochloride, and cellulose (*Nicotiana tobacum*) that were chosen as they represent commons sources inputted in acid soils by plant residues and fungus debris. Here we classify the applied substrates as readily useful for glucose and glucosamine and slightly useful for cellulose based on previous microbial utilization in soils (Gunina and Kuzyakov, 2015; Hill et al., 2008; Roberts and Jones, 2012). Also, we considered the glucosamine a slightly more complex substrate than glucose due to the amino group ($-NH_2$) in its molecular structure. So, in terms of increasing the gradient of size/complexity molecule we have glucose, glucosamine and cellulose as substrates. Finally, independent assays were carried out for each ^{14}C-labelled substrate.

Before applying the ^{14}C-labelled substrates, soil samples were submitted to pre-incubation to restore the microbial activity and avoid initial release of CO_2 biased by soil rewetting (Haney et al., 2004). Therefore, soil samples were rewetted at 50% of WHC and left open in the dark at 25 °C for 72 h as described previously. After pre-incubation, 0.5 mL of 10 mM solution containing glucose, glucosamine hydrochloride or cellulose at specific activity of 1.8 kBq mL^{-1} were uniformly added in the soil and a 6-mL vial containing 1 mL of 1 M NaOH was trapped into the tube and immediately capped. Inside the closed tube, the CO_2 released from soil diffuses into the alkaline solution as a *diffusion system*. The systems were left into the incubator in the dark at 25 °C and the traps were changed at 0.5, 1.5, 3, 5, 8, 12 and 24 h and then at 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 22, 26, 31, 37, 44, 52 and 60 d after initial soil ^{14}C-labelling. Empty tubes were also left in the incubator and changed at the same times as blank samples. After each trap change, the replaced vials were added of 4 mL of Optiphase HiSafe 3 scintillation fluid (PerkinElmer Inc., Waltham MA USA), capped, vortexed and the ^{14}C activity measured in a Wallac 1404 liquid scintillation counter (Wallac EG&G, UK). Just after removing the traps, at 60 days, the assays were ended by extracting the ^{14}C-labelled substrates remaining in soil solution and adsorbed to the soil solid phase. The samples were extracted adding 0.5 M K_2SO_4 to the soil at a 1:5 ratio (w/v) as described above. Finally, the soil extracts were readily measured for ^{14}C activity

mixing 1 mL of their aliquot with 4 mL of scintillation fluid and for ammonium and nitrate following the colorimetric methods described previously.

4.2.4 Priming kinetics induced by ^{14}C-labelled glucose reapplication upon nutrient availability

This experiment was performed using ^{14}C-labelled glucose as it represents a model substrate in which may be used by whole soil microbial community and is fully processed as CO_2 or assimilated into microbial biomass since it is weakly adsorbed to the soil solid phase (Hill et al., 2008). The soil microbial community responses to successive application of ^{14}C-labelled glucose were evaluated measuring the release of $^{14}CO_2$ during incubation time. To decouple the $^{14}CO_2$ released from successive application of ^{14}C-labelled glucose, the experiment was mirrored in which unlabelled glucose followed by ^{14}C-labelled glucose and ^{14}C-labelled glucose followed by unlabelled glucose were applied in replicated samples and the trapped $^{14}CO_2$ measured without overlapping their background (Fig. 1 upper). Complementary, an additional nutrient enrichment treatment (NPS) involving nitrogen (N), phosphorus (P) and sulphur (S) was added along with ^{14}C-labelled or unlabelled glucose in the substrate reapplication to understand the fate of ^{14}C on priming effect upon nutrient availability (Fig. 1 lower).



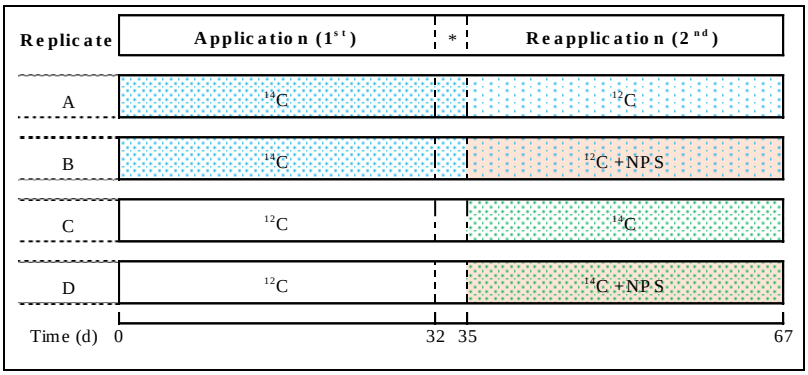


Fig. 1. Representative scheme of applications on priming effect experiment. Above: mirrored experiment for decoupling $^{14}CO_2$ released from successive ^{14}C-labeled glucose applications in soil replicates. Blue and green lines are the $^{14}CO_2$ released from 1st and 2nd applications, respectively. Below: additional nutrient enrichment treatment involving nitrogen (N), phosphorus (P) and sulphur (S) in reapplication (2nd) with labelled or unlabelled glucose (C). *: is the moisture loss period, blue and green points represent the soil samples labelling. Note the blue points density decrease from 1st to 2nd application that represent isotopic dilution.

In the laboratory, four replications of 5 g of dried soil were disposed into 50 mL-centrifuge tubes and left for pre-incubation as previously described followed by application of 0.5 mL of 10 mM glucose solution labelled or not at specific activity of 1.8 kBq mL^{-1} resulting in two sets of labelled replicates while two sets remained unlabelled (Fig. 1 upper). Just after soil labelling, a 6-mL vials containing 1 mL of 1 M NaOH was trapped into each tube and capped. The unlabelled soil samples were covered with paraffin film (Parafilm® M) to keep their moisture while allowing gas exchanges during the incubation period. Trap changes occurred at 1, 3, 6, 12 and 24 h and then at 2, 3, 4, 5, 7, 10, 14, 19, 25, and 32 d after initial soil ^{14}C-labelling. At 32 d, the daily rate emissions decreased (< 0.3% of total applied) leading the evolved $^{14}CO_2$ to stabilization, that is, every labile ^{14}C was released as $^{14}CO_2$ and just non-labile ^{14}C assimilated by microbial biomass remained; at this point, all samples were left uncovered and exposed to air-drying in the dark at 25 °C for 72 h allowing moisture loss down to 25% of WHC. Thereby, we support the association of priming effect with substrate (+NPS) reapplication at all instead of a dry/rewet effect (Haney et al., 2004) or a methodological artefact (Kuzyakov, 2010). To induce the priming effect during the unlabelled glucose reapplication and to relate the CUE under successive glucose application while considering the NPS effects, four different glucose solutions were setup: ^{14}C-labelled glucose, ^{14}C-labelled glucose + NPS, unlabelled glucose and unlabelled glucose + NPS (Fig. 1 lower). The glucose content and specific activity (when ^{14}C-labelled) followed the same patterns as in the first application (10 mM and

1.8 kBq mL^{-1}) and the NPS involved the addition of NH_4NO_3-N, $(NH_4)_2HPO_4$-P and $(NH_4)_2SO_4$-S assuming a microbial biomass C:N:P:S ratio of 250:8.75:1.25:1 (pH 6.5) based on the study of Creamer et al. (2014). At 35 d, 0.5 mL of the glucose solutions were reapplied while bringing the soil moisture to 50% of WHC and a 6-mL vial containing 1 mL of 1 M NaOH was trapped into the tube and capped. The trap changes occurred at the same frequency as the first application up to 67 d. All trap changes and their measurements followed the same procedure described previously.

4.2.5 Rationale for modelling and calculations

Traditionally, first-orders reactions have been widely used for modelling the kinetic of soil organic matter decomposition at all. In such reactions, an initial source of organic matter (P_0) decreases its content ($C(t)$) along some period (t) proportionately to a decay constant (k) in which can be described in a reduced integrated form as a monophasic exponential decay equation:

$$C(t) = P_o \times exp^{-k \times t} \quad \text{(Eq. 1)}$$

As the organic matter comprises a multitude of pools with their respective sizes and specific reactions on soil it is inherent for kinetic modelling their splitting in discrete pools to bring up a general simplified equation for a multiphasic pattern of first-order exponential decay reactions. However, considering the inability to identify and isolate the existing range of pools in the soil organic matter several models have been proposed to describe the substrate-derived C dynamics in soil (Saggar et al., 1996; Toal et al., 2000; Van Hees et al., 2005), each of them with their worth but also supposing a known constraints on it. Therefore, the combination of first-order exponential decay equation and theoretical background on soil C dynamics have been provided useful models to predict pools, decay rates and carbon use efficiency (CUE).

According to Glanville et al. (2016), the most granted models underlying studies in soil substrate decomposition presents two or three phases of first-order exponential decay reactions in which the right-term side of the Eq. 1 is replicated in the respective number of phases resulting in the double (C_2) and triple (C_3) exponential decay models (EDM): $C_2 = C_{1.2} + C_{2.2}$ and $C_3 = C_{1.3} + C_{2.3} + C_{3.3}$. In general, short-term incubation assays using labelled substrates are usually fitted in the double EDM (few hours to

couple of days) (Hill et al., 2008) while longer assays in the triple EDM (Farrar et al., 2012). However, that also depends on data scatter. Technically, the first phase of decay from both models ($C_{1.2}$ and $C_{1.3}$) resembles cause the C transformations follow attached to the fast-initial decreasing of ^{14}C remaining in soil in which it is easily detected and split from the rest of the data by any parameter optimizer method. Usually, this decay phase is known as *fast* pool and assumed to represent catabolic process (i.e., maintenance-derived respiration), whilst some author also includes anabolic process such as cell growth depending on the labile-C content and availability (Scow et al., 1986). The remaining decay phases at both models ($C_{2.2}$, $C_{3.2}$ and $C_{3.3}$) are attached to the residual data (original data subtracted from estimated data of the first phase) in which must follow the exponential decay left over. At this point, the second decay phase of the triple EDM ($C_{2.3}$) is identified as *slow* pool and related to the C temporarily immobilized in the microbial biomass while its third decay phase ($C_{3.3}$) has a very slow C decomposition pattern (*passive* pool) being related to the degree of biochemical (i.e. humified) and/or physical (i.e. occluded) protection/stability in soil (Farrar et al., 2012). Occasionally, the incubation time and data density can be insufficient and the exponential decay pattern of the residual data of the triple EDM fails resulting an overlapped information of $C_{2.3}$ and $C_{3.3}$ extracted from the model. In that cases, the triple EDM should be reduced to double EDM and so its second phase of decay ($C_{2.2}$) can be promptly estimated being nearly equivalent to the match of *slow* and *passive* pools (Motavalli et al., 1994). As consequence, the liability of the connectivity between the remaining decay phases (i.e. $C_{2.2}$, $C_{2.3}$ and $C_{3.3}$) and the limited understanding of their extensions into the model have been supporting studies to calculate substrate half-times only for the *fast* pool (Boddy et al., 2007; Farrar et al., 2012). Here, the double and triple EDM phases were defined as follow:

$$C_{1.3}(t) = P_{1.3} \times exp^{-k_{1.3} \times t} \quad \text{(Eq. 2)}$$

$$C_{2.3}(t) = P_{2.3} \times exp^{-k_{2.3} \times t} \quad \text{(Eq. 3)}$$

$$C_{3.3}(t) = P_{3.3} \times exp^{-k_{3.3} \times t} \quad \text{(Eq. 4)}$$

$$C_{1.2}(t) = P_{1.2} \times exp^{-k_{1.2} \times t} \quad \text{(Eq. 5)}$$

$$C_{2.2}(t) = P_{2.2} \times exp^{-k_{2.2} \times t} \quad \text{(Eq. 6)}$$

where *C* is the cumulative amount of substrate-derived ^{14}C remaining in soil (%) at time *t*, *P* refers to initial pool sizes as cumulative amount of substrate-derived ^{14}C remaining in soil (%), *k* refers to the decay constants (d^{-1}), *t* is the incubation time (*d*) and the numeric subscripts *1*, *2* and *3* on the left-term side refer to first, second and third phases of the EDM while the numeric subscripts *2* and *3* on the right-term side refer to double and triple EDM.

In our study, the wide heterogeneity of soil treatments (i.e., sites/textures, layers, systems) and their interaction with additional factors as organic and inorganic substrates (re)application became impracticable to represent all the data scatter and decay patterns of substrate-C in a single and robust model. In fact, at some situations an evident initial lag-phase were detected (e.g., subsoil treatments under ^{14}C-laballed glucose and glucosamine). According to Gillis and Price (2011), initial lag-phases in substrate decay models could be assigned to sigmoidal decays that would represent a C pool not readily mineralized to a large extent by microorganisms. That is because sigmoidal decay models (SDM) have biological interpretation describing the accumulated growth of (micro)organisms. However, it does not mean that the entire sigmoidal pool must be carried out under anabolic process. Instead, both exponential and sigmoidal reactions upon *fast* pools combine a mix of metabolic processes (Parton et al., 2015) and supported the substitution of the first phases of decay from exponential ($C_{1.3}$ and $C_{1.2}$) to sigmoidal equation ($C_{1.3s}$ and $C_{1.2s}$) without losing the relationship between pools intra and inter models whenever found a predominant sigmoidal shape in the data scatter. Here, the double and triple SDM were defined as follow:

$$C_{1.3s}(t) = \frac{P_{1.3}}{1+exp^{-k_{1.3s}\times(t-T)}} \quad \text{(Eq. 7)}$$

$$C_{1.2s}(t) = \frac{P_{1.2}}{1+exp^{-k_{1.2s}\times(t-T)}} \quad \text{(Eq. 8)}$$

where C_s is the cumulative amount of substrate-derived ^{14}C remaining in soil following the sigmoidal decay (i.e. inverted sigmoidal function) (%) at time *t*, *P* refers to initial pool sizes as cumulative amount of substrate-derived ^{14}C remaining in soil (%), k_s refers to the decay constant and thus with negative values (d^{-1}), *t* is the incubation time (d), *T* indicates the time that *k* is maximum, and the numeric subscript *1* on the left-term side refers to first phase of the SDM while the numeric subscripts *2* and *3* on the

right-term side refer to double and triple SDM. Note that $P_{1.2}$ and $P_{1.3}$ were the only parameter with the same meaning for exponential and sigmoidal equations and so could be related between them.

The data from substrate-derived ^{14}C remaining in soil were individually tested in the models above mentioned using *nlme* package and applying the *gnls* function in R (Pinheiro et al., 2018). Data scatters with predominant sigmoidal shape were attached for SDM. The model fitting involved a quasi-Newton method optimizer to estimate the initial parameters by decreasing the mean square error to the minimum with correlation structure through temporal dependencies of the errors (*correlation* argument). The models were fitted by each replication and based up on two assumptions: the model should give parameters with biological explanations following classical decay studies (Motavalli et al., 1994; Scow et al., 1986; Van Veen and Paul, 1981), and; the model parameters should be significant to it at all ($p < 0.100$). The last was fundamental to keep the pools and parameters of the models without resulting in lack of fit. We did not present the coefficient of determination (r^2) cause its use is exclusive for linear models fit.

Due to the large number of comparisons and the meaningless of extracting the mean of the parameters by factor (*system* and *soil layer*) we considered the soil microbial CUE or just CUE as a proxy for treatments comparison. The CUE is commonly defined as the ratio of the stabilized C and the total taken up by microbial biomass. Here, the estimated pools were used for CUE calculation as follow:

$$CUE_3 = \frac{P_{3.3}}{P_{1.3}+P_{2.3}+P_{3.3}} \quad \text{(Eq. 9)}$$

$$CUE_2 = \frac{P_{2.2}}{P_{1.2}+P_{2.2}} \quad \text{(Eq. 10)}$$

where *CUE* is the carbon use efficiency (%) and the numeric subscripts *2* and *3* refer to double and triple EDM or SDM.

All data were presented as mean value followed by standard error of the mean (SEM). The data residues were tested for normality distribution and homoscedasticity using Shapiro-Wilk's test and Bartlett's test ($p < 0.100$) respectively. The data from (bio)chemical analyses and estimated parameters were inputted in a statistical model with *system* and *soil layer* considered as fixed factors under complete randomized block design with spatial repeated-measures (*system*layer* + *block/system*) following

ANOVA ($p < 0.100$). For the substrates decomposition and priming assays, the data from CUE were inputted in a similar statistical model but added respectively by the factors *substrate* and *application* (*system*layer*substrate* + *block/system/layer* and *system*layer*application* + *block/system/layer*). Even relating Oxisols, *site* was not considered into the statistical models because of the impossibility to ascribe their edaphoclimatic characteristics (i.e., soil texture, climatic conditions) to the results. We considered that there was no spatial dependence between top- and subsoil data because the layers were not subjacent and so spatially disconnected. When the *F*-test showed significance, Tukey's HSD test was applied to identify the differences between the means of the treatments ($p < 0.100$). All the interactions were showed for the estimated parameters into *site* for each substrate regardless of *F*-test significance because the average of the factors by *system* and *soil depth* distorted the meanings for their models. All statistical analyses and graphs were performed in R (R Core Team, 2019).

4.3 Results

4.3.1 C and N forms in soil

Overall, system differences were detected in topsoil while just a few in subsoil. In topsoil, total C and N were higher in ES than SA with IA as transient system in which larger differences were found at PG site. The dissolved organic forms of C and N followed the results obtained in their total content but with larger differences between systems at IT site (at least two-fold more). Also, the DOC and DON at IA system was closely related to SA mainly at IT site. In subsoil, no difference was found in TOC and TN while DOC and DON remained higher in ES system mainly at PG site. As expected, all topsoil systems had higher content of total and dissolved forms of C and N than in subsoil. On the other hand, sparse differences were found at C and N microbial biomass at PG while not following the results in the dissolved forms (Fig. 2).

The soil extraction for DIN determination after substrates incubation showed similar differences as obtained in DON but on a large scale. The differences inside substrate were higher in ES than in agrosystems (Fig. 3 lower). On average, DIN content on topsoil were three- (IT) and six-fold (PG) more than subsoil and cellulose substrate produced the higher DIN content in both sites (Fig. 3 upper). In general, DIN content after incubation were higher than prior to incubation. Furthermore, compared to extraction prior to incubation an increasing of up to 119% (IT) and 23% (PG) were

detected on topsoil systems after incubation while low increments were detected on subsoil (Fig. 4).

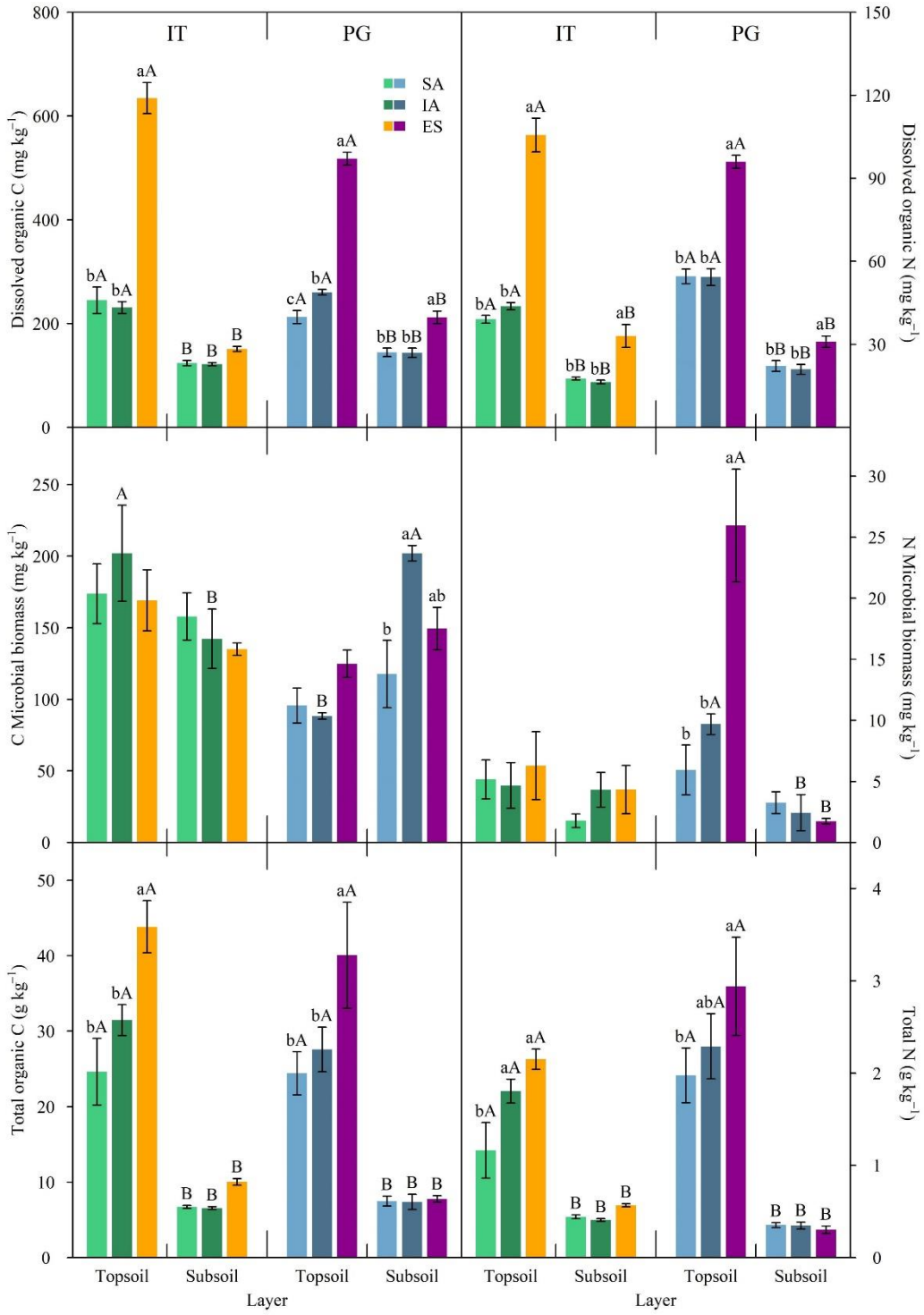


Fig. 2. Effect of system and soil layer on dissolved organic carbon (DOC) and nitrogen (DON), carbon (CBM) and nitrogen (NBM) microbial biomass and total organic carbon (TOC) and nitrogen (TN). IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. Vertical bars represent treatment means ± SEM (n

= 4) of the measured replicates. For interactions split into sites, lowercase letters indicate significant differences among systems within soil layers while uppercase indicate significant differences among soil layers within systems

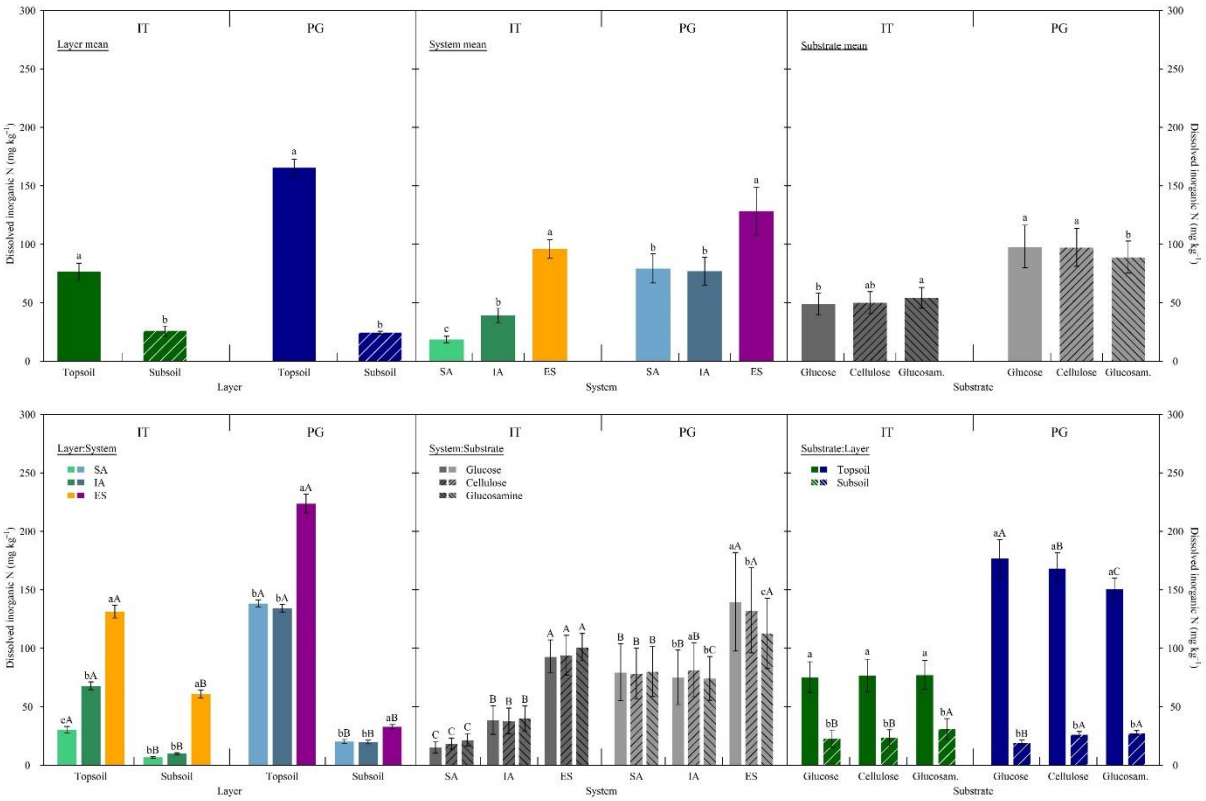


Fig. 3. Effect of system, soil layer and substrates on dissolved inorganic nitrogen (DIN). Vertical bars represent treatment means ± SEM (n = 4) of the measured replicates. IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. Upper graphics present the average of each factor and the lower graphics present interactions in each two of three factors. For interactions split into sites, lowercase letters indicate significant differences among treatments with legend within each treatment under x-axis while uppercase indicate significant differences among treatments with the same legend between treatments under x-axis

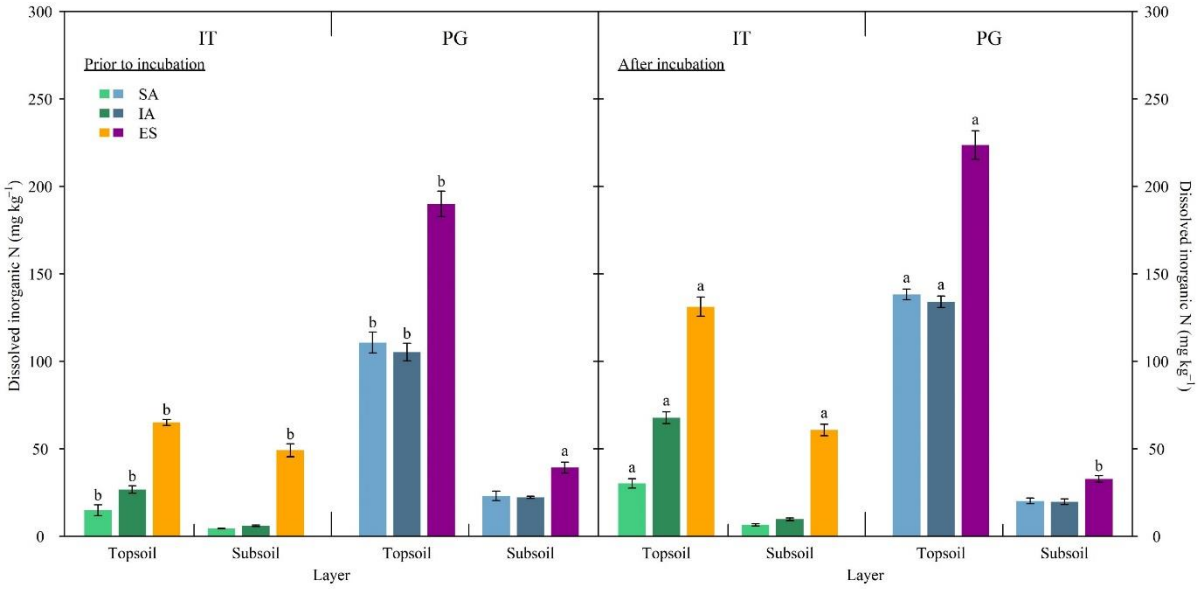


Fig. 4. Effect of incubation on N mineralization. IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. Vertical bars represent treatment means ± SEM (n = 4) of the measured replicates

4.3.2 Kinetics of decomposition, parameters, and CUE of discrete substrates

The most contrasting difference on decomposition pattern after substrates application were detected in the soil layers. Glucose and glucosamine presented a lag-phase in subsoil treatments. In that substrates, the kinetics of decomposition were modelled applying EDM and SDM in the top- and subsoil treatments respectively (Fig. 5 and 7). On average, the subsoil systems exhibited a lag-phase with *T* three-fold smaller and k_{1s} four-fold lower in glucose than in glucosamine. The lag-phase was not detected in cellulose treatments so the EDM could be applied in the soil layers at all (Fig. 6).

In the model fittings, exponential or sigmoidal decays, the higher the fast pool sizes ($P_{1.2}$ or $P_{1.3}$) lower the passive pool sizes ($P_{2.2}$ or $P_{3.3}$). In glucose and cellulose, the fast pool sizes in topsoil systems were superior to subsoil while the inverse occurred in the passive pool sizes. For glucose, the agrosystems had similar decomposition patterns but distinct from ecosystems. ES showed the most contrasting system in which the fast pool size decreased from 55% in topsoil to 17% in subsoil at IT site and from 38.3% in topsoil to 25.6% in subsoil at PG site while the passive pool size increased from 35.6% in topsoil to 65.2% in subsoil at IT site and from 39% in topsoil to 39.2% in subsoil at PG site (Suppl. Table 2 of the Appendix). In cellulose treatments, although without lag-phase, the decomposition pattern in subsoil systems were less pronounced related to topsoil. The fast pool sizes in subsoil systems were

at least five- and two-fold lower than in topsoil while the passive pool sizes increased on average 25.9% and 32.2% at IT and PG site respectively (Suppl. Table 3 of the Appendix). On the other hand, the double decay pattern in glucosamine treatments showed opposite contribution in the fast and slow + passive pool sizes of topsoil at IT and PG sites. Also, the fast pool at IT treatments were lower than in PG site (Suppl. Table 4 of the Appendix).

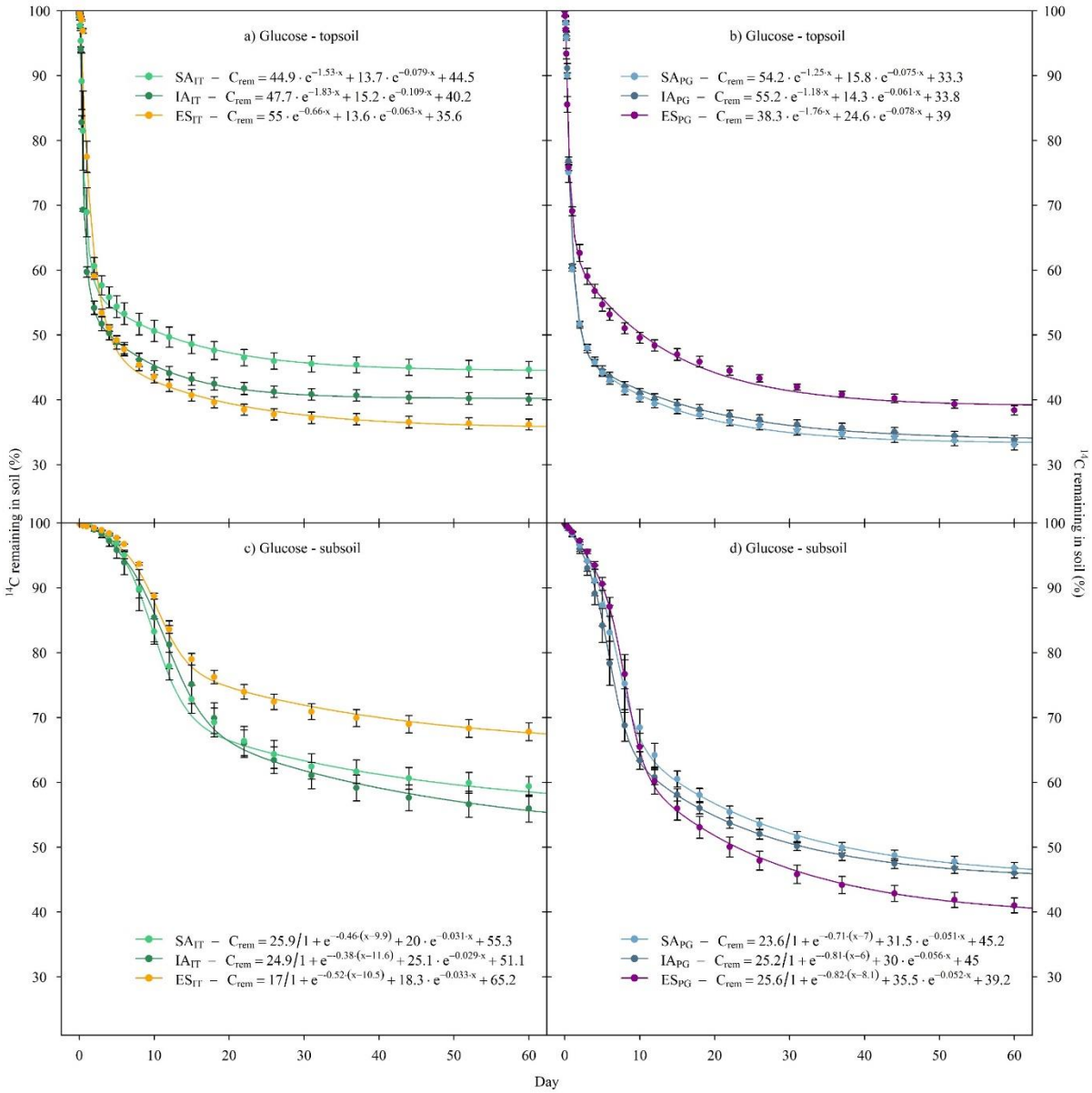


Fig. 5. The percentage of glucose-derived ^{14}C remaining in soil during the incubation at different treatments (colors), sites and soil layers

(panels a–d). IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. Full circles represent means ± SEM (n = 4) of the measured replicates while the curves were the fitted models based up on the average of the curves fit (triple exponential decay model at 5 parameters for topsoil and triple-lag exponential decay model at 6 parameters for subsoil treatments). The equations are shown on top of the panels and the statistics of their parameters are in Suppl. Table 2 (Appendix)

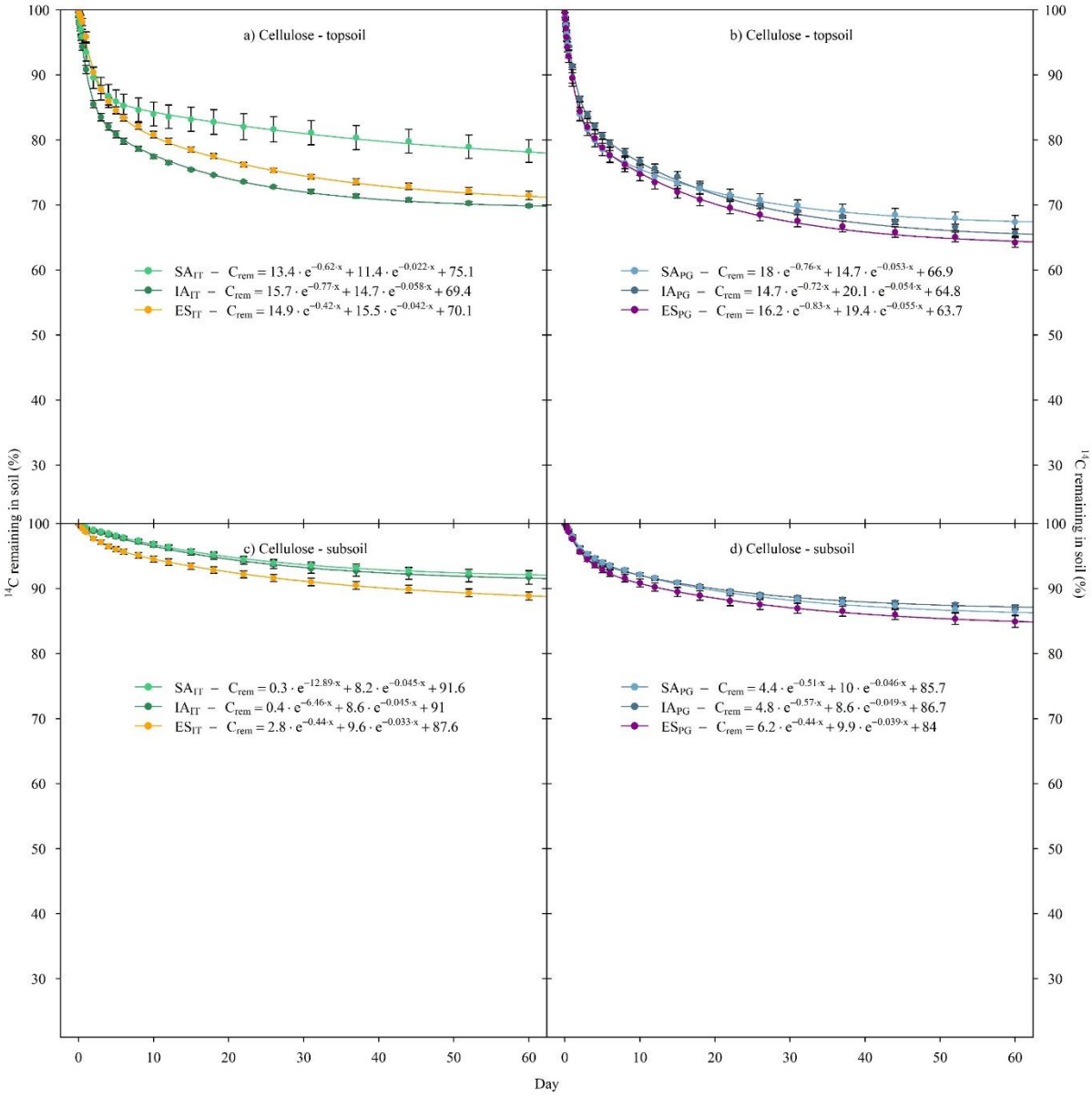


Fig. 6. The percentage of cellulose-derived ^{14}C remaining in soil during the incubation at different treatments (colors), sites and soil layers (panels a–d). IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. Full circles represent means ± SEM (n = 4) of the measured replicates while the curves were the fitted models based up on the average of the curves fit (triple exponential decay model at 5 parameters for topsoil and subsoil treatments). The equations are shown on top of the panels and the statistics of their parameters are in Suppl. Table 3 (Appendix)

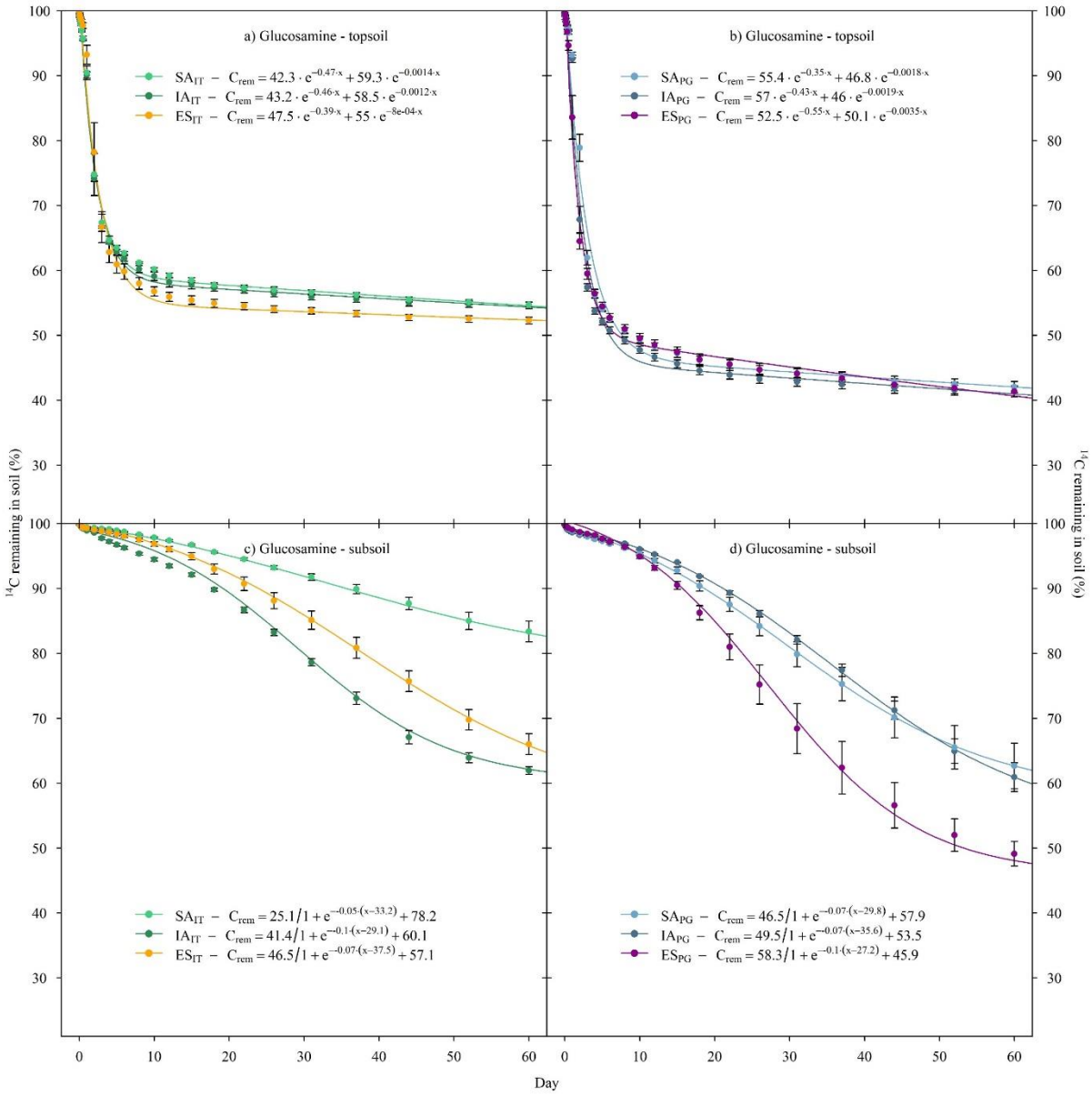


Fig. 7. The percentage of glucosamine-derived ^{14}C remaining in soil during the incubation at different treatments (colors), sites and soil layers (panels a–d). IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. Full circles represent means ± SEM (n = 4) of the measured replicates while the curves were the fitted models based up on the average of the curves fit (double exponential decay model at 4 parameters for topsoil treatment). No meaningful model could be fitted in the subsoil data due to their lack to reach an asymptote of stabilization. The equations are shown on top of the panels and the statistics of their parameters are in Suppl. Table 4 (Appendix)

In general, the decomposition pattern and so the parameters between site, soil layer and systems in each substrate varied widely. Also, it was complex to relate a decomposition pattern between sites, systems, and substrates, although relatively simple between soil layers. So, since we could not extract the mean of the parameters

by factor (*system* and *soil layer*) because of the distortion on their meanings and for their whole models we considered the CUE as a proxy for treatments comparison. The subsoil had higher efficiency on stabilizing C than in topsoil from both sites. On average, the CUE on subsoil were 70% and 59.6% against 55.6% and 48.8% on topsoil respectively at IT and PG (Fig. 8 upper). These results were also extended within all systems and substrates treatments showing the consistency on the CUE differences between soil layers. Within soil layer, the SA and cellulose treatments presented wider differences between sub- and topsoil, respectively 14.2% and 19.2% and so the better gap upon increasing the CUE (Fig. 8 lower).

On average, the SA were 5.6% (IT) and 2.2% (PG) superior on CUE than their respective ecosystems (Fig. 8 upper). These differences remained more pronounced in subsoils but not repeated in topsoil at PG site. Inside systems, greater results were obtained by application of cellulose and glucosamine in SA showing the efficiency of 83.5% and 67% for IT and 76.4% and 50.6% for PG; glucose had no differences between systems at PG. Interestingly along the systems comparison, the IA have been positioned in a transition point between the SA and ES at both sites, the same as found for total C and N (Fig. 8 lower).

Substrate treatments showed a clear positive relation between the CUE and molecular size/complexity. On average, the CUE was 47.7%, 59.9% and 80.8% at IT and 38.7%, 48.4% and 75.4% at PG respectively for glucose, glucosamine, and cellulose, with IT the most potential site to the substrate effects (Fig. 8 upper). This effect repeated on each level of substrate interaction within the other factors, with cellulose application in subsoil systems at IT the most promisor treatments on increasing the CUE.

The CUE in all treatments at IT were higher than at the PG site although that factor was not inside de statistical model; on average, the CUE at IT was 8.6% higher but reached around 11% into subsoil, SA and glucosamine treatments (Fig. 8 upper).

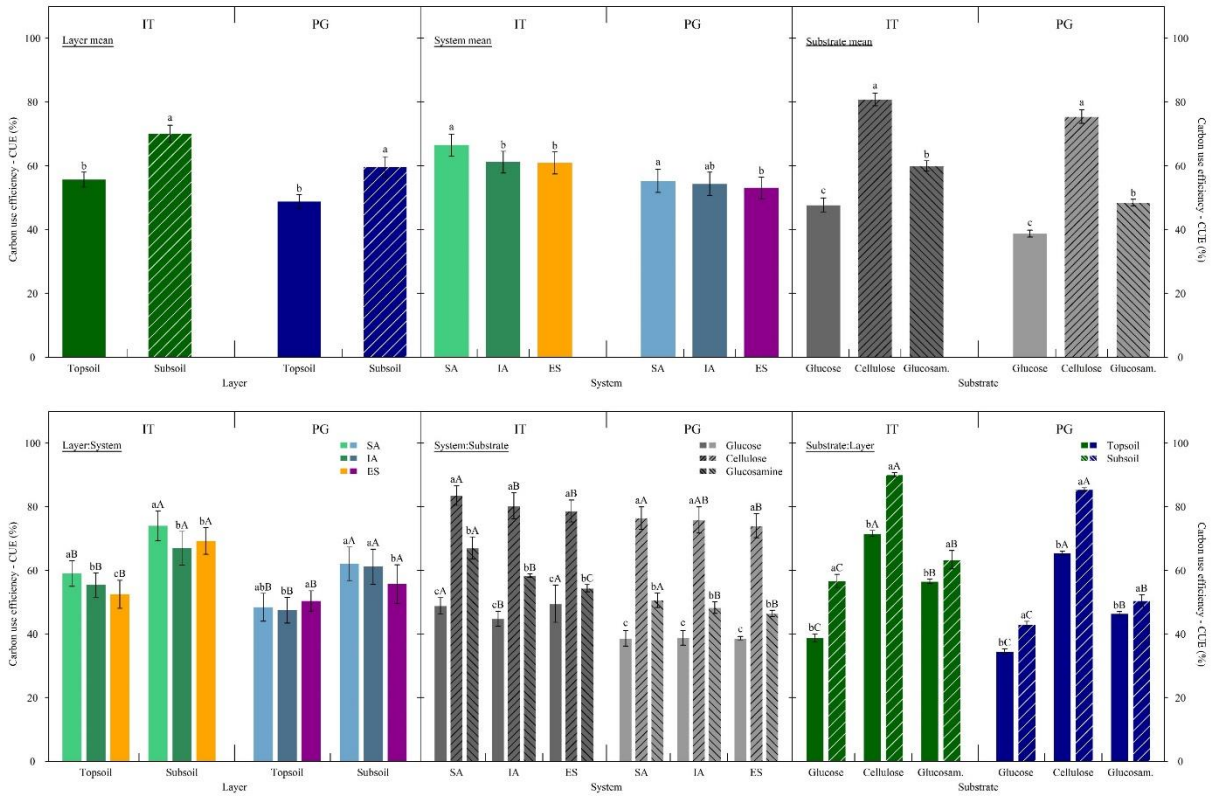


Fig. 8. Effect of system, soil layer and substrates on carbon use efficiency (CUE). Upper graphics present the average of each factor and the lower graphics present interactions in each two of three factors. IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. Vertical bars represent treatment means ± SEM (n = 4) of the measured replicates. For interactions split into sites, lowercase letters indicate significant differences among treatments with legend within each treatment under x-axis while uppercase indicate significant differences among treatments with the same legend between treatments under x-axis

4.3.3 Priming kinetics and CUE affected by glucose reapplication upon nutrient availability

This experiment approached two different glucose assays (mirrored) under different period time and evaluation frequency for priming approaching. To compare substrates, the glucose assay reached 60 days with 25 evaluations while 32 days with 16 evaluations for priming assay in the 1st application. Although the decomposition patterns of glucose assays were similar, the parameters were slightly different from each other, mainly in subsoil treatments that were modelled by applying the double SDM instead of triple SDM used under 60-days assay (Fig. 5 and Fig. 9). As we expected, the reapplication of unlabelled glucose at 35 d promoted a break in the decomposition curve of ^{14}C-labelled in the 1st application where a new curve derived from priming decomposition was fitted (Fig. 9).

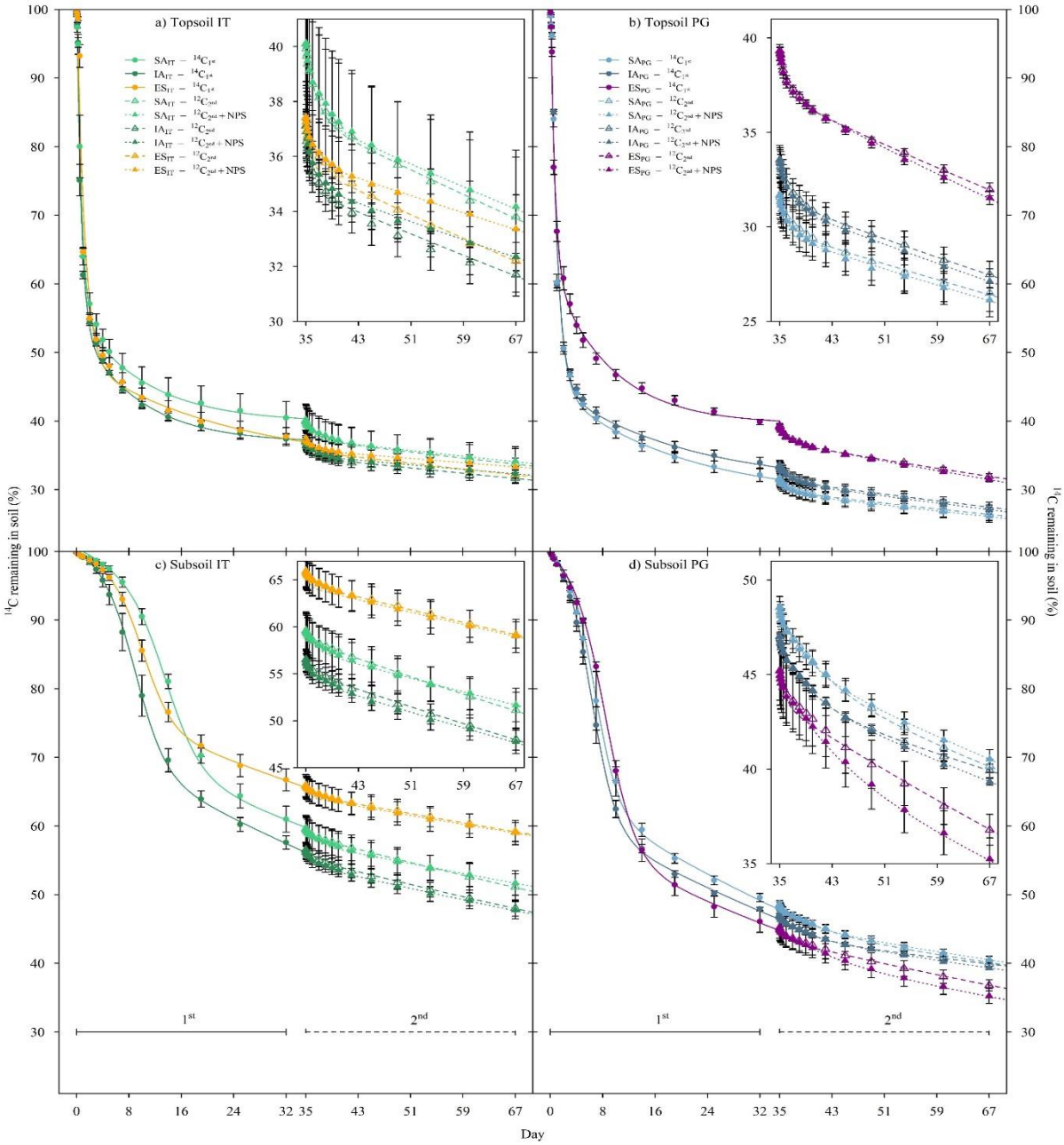


Fig. 9. The percentage of ^{14}C remaining in soil during successive incubation periods at different treatments (colors), effect of solutions applications (line type), site and soil layers (panels a–d). IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. The application of ^{14}C-labelled glucose solution (1st) involved the period incubation from 0 to 32 d while the reapplication (2nd) in the same replicates of a priming unlabelled glucose solution enriched or not in nutrients (NPS) involved the period incubation from 35 to 67 d. The period between 32 and 35 d were saved for the moisture loss necessary before the reapplication. Points characters represent means ± SEM (n = 4) of the measured replicates while the curves were the fitted models based up on the average of the curves fit (triple exponential decay model at 5 parameters for topsoil and double-lag exponential decay model at 5 parameters for subsoil treatments during 1st application, and double exponential decay model at 4 parameters for all treatments during 2nd application). The inset graphs provide a better resolution view of the immediate priming response. Note different y-axis scales for inset panel graphs

In all treatments the double EDM had a better fit model and so facilitating the comparison and interpretation of the parameters. The fast pool size from that curves was considered as the result from priming effect. On average, $P_{1.2}$ from top- and subsoil were 2.0% and 1.4% at IT and 2.0% and 2.9% at PG. Regarding nutrient enrichment, its effect was detected only in topsoil of IT treatments. Furthermore, the priming decomposition pattern after unlabelled glucose applications showed the disappearance of the lag-phase in subsoils treatments (Fig. 9).

We considered the CUE as a proxy for measuring the priming effect upon nutrient availability as well. As the unlabelled glucose reapplication was performed and a new $P_{1.2}$ came through, the CUE clearly decreased. The priming effect on CUE were widely detected inside soil layers and systems. On the other hand, nutrient enrichment effect was signed at IT subsoil and in SA_{IT} and ES_{PG} subsoil. On average, only IT site showed difference between priming and nutrient enrichment (Fig. 10 upper).

Aligned to the evaluation of the priming effect, decoupling the $^{14}CO_2$ released from successive ^{14}C-labelled glucose application allowed to compare the kinetics decomposition of ^{14}C-labelled glucose at 1st and 2nd application also upon nutrient enrichment. The results revealed strong changes on decomposition patterns and their parameters at all. In general, the ^{14}C-labelled glucose at 2nd application and nutrient enrichment increased the passive pool in topsoil but decreased it in subsoil. Furthermore, nutrient enrichment enhanced the decomposition rate of the fast pool ($k_{1.3}$ and $k_{1.2}$) at 10 of 12 treatments compared to those without nutrients addition. However, the most interesting result was the migration of the decomposition pattern in subsoil from double ESM to double EDM respectively in the 1st and 2nd application of ^{14}C-labelled glucose, that is, there was no detectable lag-phase after glucose reapplication. Furthermore, it seems that nutrient enrichment contributes to this migration by favouring the exponential decay through increasing the $C_{1.2}$, mainly at IT site (Fig. 11).

The efficiency on stabilizing C in IT topsoil was higher in the 2nd application related to 1st application as consequence of increasing the passive pool size. However, the CUE in subsoil decreased at both sites following the decreasing of passive pool in the 2nd application treatments; at IT topsoil, the nutrient enrichment had even more effect on decreasing the CUE (Fig. 10 middle).

The sum of $^{14}CO_2$ evolved in the 2nd application from both unlabelled (priming) and ^{14}C-labelled glucose was compared to the at 1st application of ^{14}C-labelled glucose to relate their decomposition kinetics under accumulated (or coupled) effect of ^{14}C-labelled glucose reapplication. The results from $^{14}CO_2$ evolved in the 2nd application showed a large increasing on the passive pool compared to the 1st application, even more pronounced than the previous results (^{14}C-labelled glucose only at 2nd application). Also, the lag-phase absence presented previously in subsoil at 2nd application remained in the coupled emissions (Fig. 12). On average, coupled emissions at 2nd application had better CUE than in 1st application lonely. The CUE under glucose reapplication within system were higher than at 1st application of both sites but no differences were found for nutrient enrichment (Fig. 10 lower).

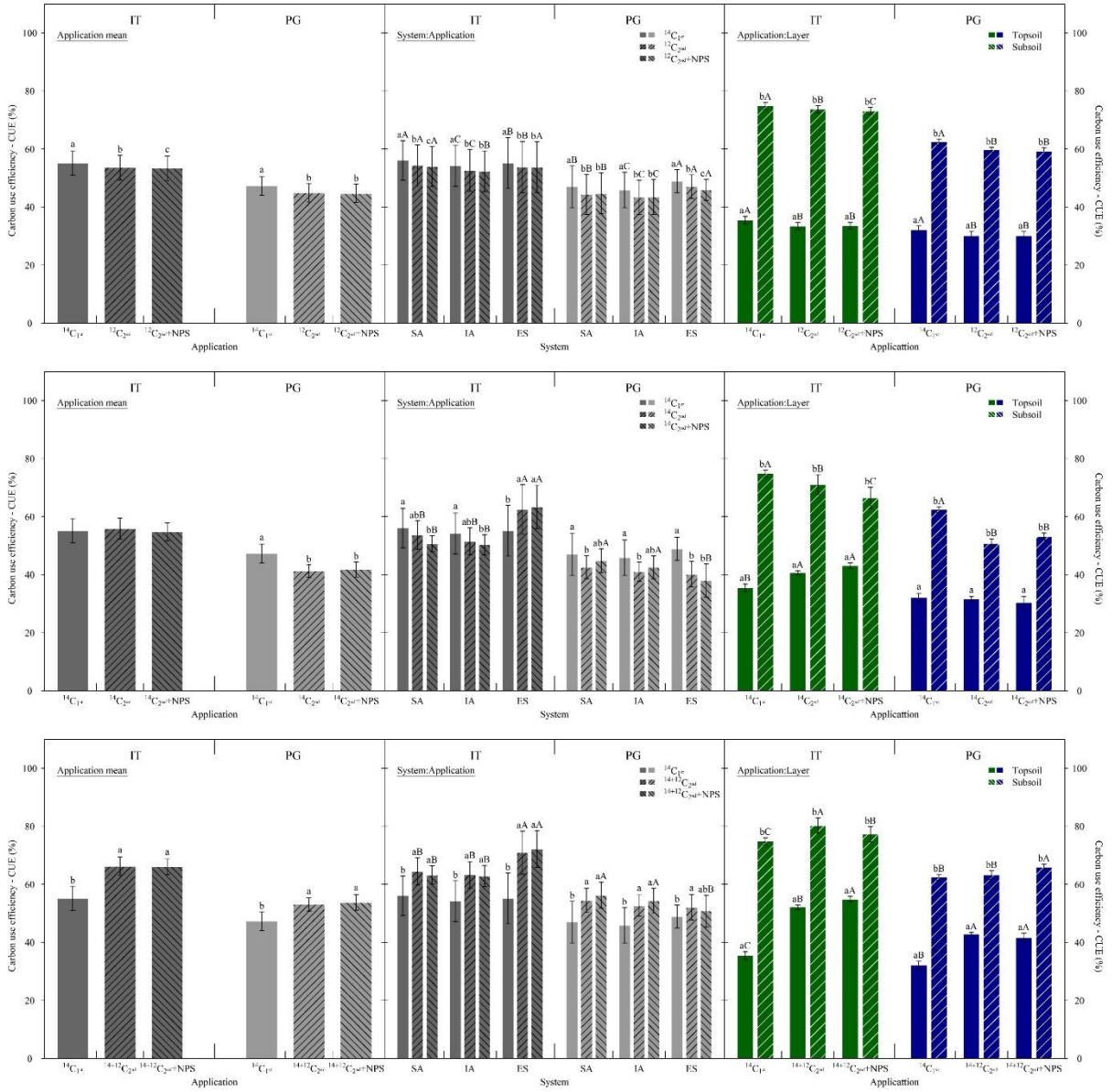


Fig. 10. Effect of system, soil layer and application on carbon use efficiency (CUE). IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. The graphics show the average of application factor and the interactions system*application and application*layer considering that: upper graphics present the comparison of ^{14}C at 1st application and following priming effect after ^{12}C at 2nd application upon nutrient availability; middle graphics present the comparison of ^{14}C at 1st application and ^{14}C at 2nd application upon nutrient availability; lower graphics present the comparison of ^{14}C at 1st application and following priming effect after ^{12}C + ^{14}C at 2nd application upon nutrient availability. Vertical bars represent treatment means ± SEM (n = 4) of the measured replicates

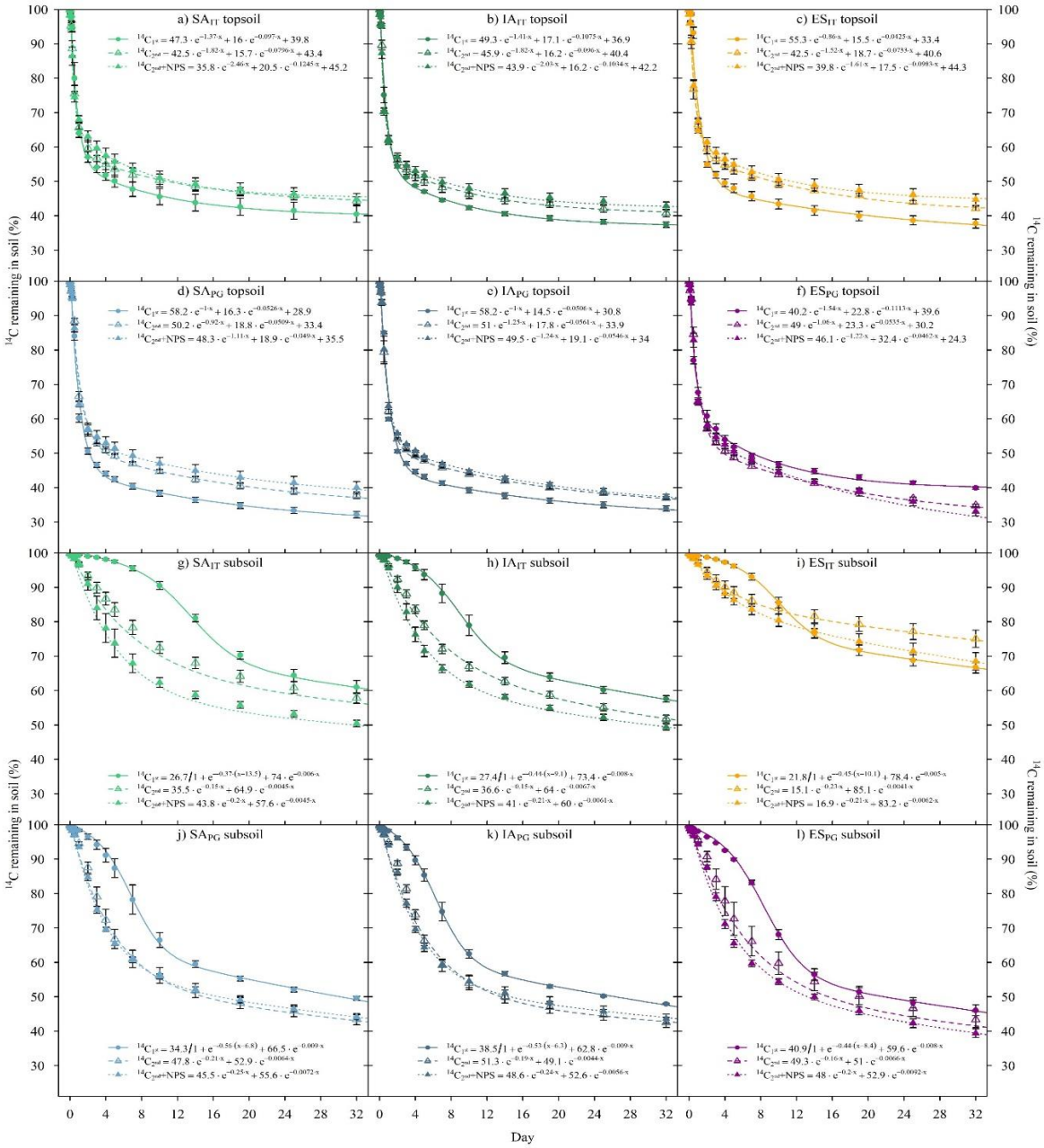


Fig. 11. Influence of successive application of ^{14}C-labeled glucose solution on different sets of soil replicates represented as the percentage of ^{14}C remaining in soil during successive incubation periods at different treatments (colors), effect of solutions applications (line type), site and soil layers (panels a–l). IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. The application of 14C-labeled glucose solution (1st) to one set of soil replicates involved the period incubation from 0 to 32 d while the reapplication (2nd) of 14C-labeled glucose solution enriched or not in nutrients (NPS) to another set of soil replicates involved the period incubation from 35 to 67 d. The period between 32 and 35 d were saved for the moisture loss necessary before the reapplication. Points characters represent means ± SEM (n = 4) of the measured replicates while the curves were the fitted models based up on the average of the curves fit (triple exponential decay model at 5 parameters for topsoil treatments during 1st and 2nd application, double-lag exponential decay model at 5 parameters for subsoil treatments during 1st application, and double exponential decay model at 4 parameters for all treatments during 2nd application). The inset graphs provide

a better resolution view of the immediate priming response. Note different y-axis scales for inset panel graphs

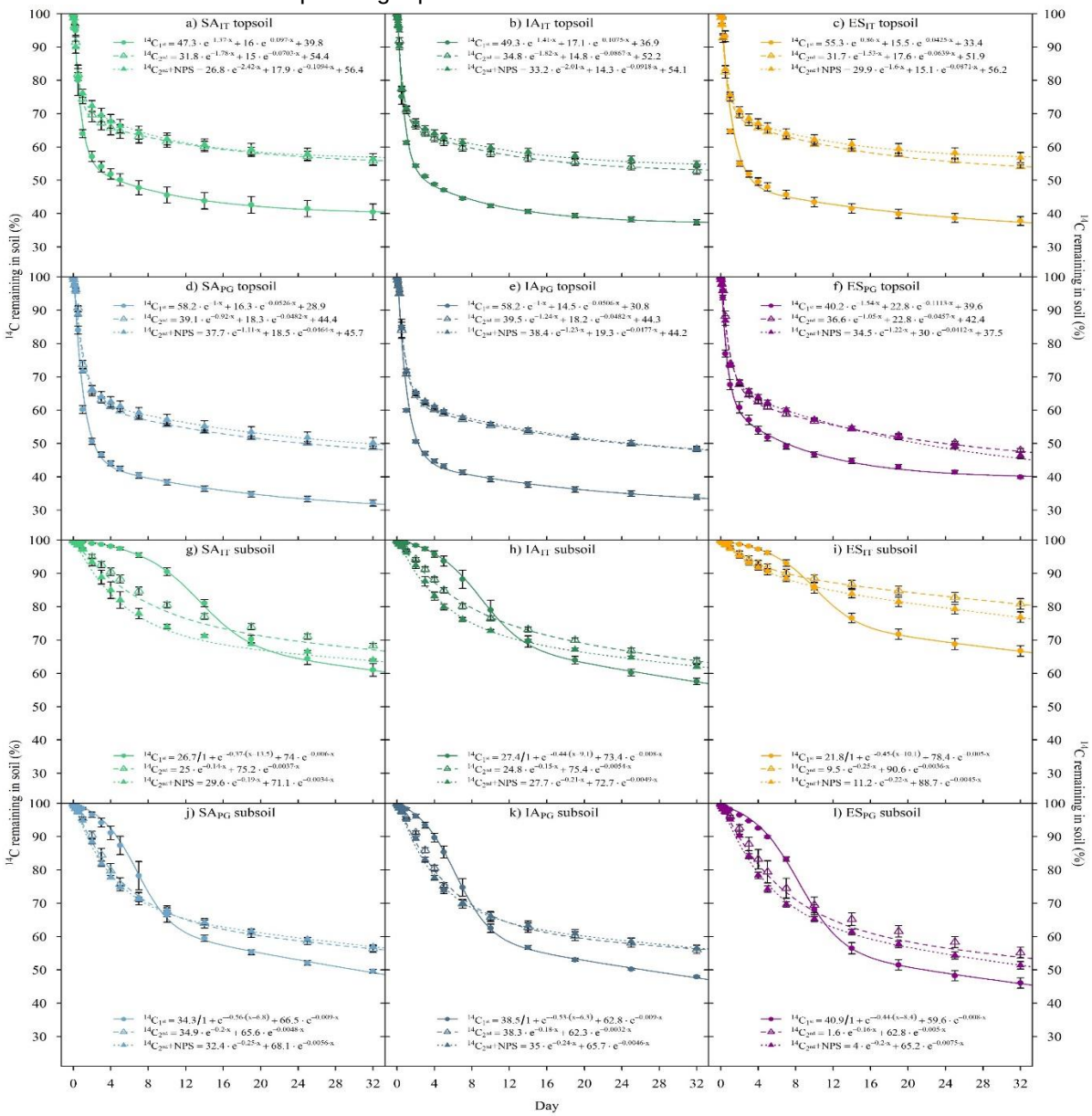


Fig. 12. Influence of successive application of ^{14}C-labeled and unlabelled glucose solution on different sets of soil replicates represented as the percentage of ^{14}C remaining in soil during successive incubation periods at different treatments (colors), effect of solutions applications (line type), site and soil layers (panels a-l). IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. The application of ^{14}C-labeled glucose solution (1st) to one set of soil replicates involved the period incubation from 0 to 32 d while the reapplication (2nd) represented the sum of the ^{14}C remaining in soil derived from the priming unlabelled glucose solution in the same replications as the 1st application with that derived from the ^{14}C-labeled glucose solution applied to different set of soil replicates, both enriched or not in nutrients (NPS) and involved the period incubation from 35 to 67 d. The period between 32 and 35 d were saved for the moisture loss necessary before the reapplication. Points characters represent means ± SEM (n = 4) of the measured replicates while the curves were the fitted models based up on the average of the curves fit (triple exponential decay model at 5 parameters for topsoil treatments during 1st and 2nd application, double-lag exponential decay model at 5 parameters for subsoil treatments during 1st

application, and double exponential decay model at 4 parameters for all treatments during 2nd application). The inset graphs provide a better resolution view of the immediate priming response. Note different y-axis scales for inset panel graphs.

4.4 Discussion

4.4.1 CUE affected by systems and soil layer

It seems that the intensification of the regional agrosystems (IA), basically by increasing diversity of crops in rotation, have been positioned the IA in a transition point between the SA and ES (Fig. 8). As we understand, the IA was a succession step towards to the sustainability of the grain production agrosystems at both sites and their level of CUE closely related to their ecosystems comply that. The explanation may be related to the intensification of the systems reducing the deficit of C saturation closer to ES and so making IA less efficient in stabilizing C in the soil (Stewart et al., 2007). Since IA and ES had similar efficiency on stabilizing C initially by microorganisms, greater efforts must be invested on the regional standard agrosystems due to their higher potential on C stabilization while maintaining the IA management in the already established areas, mostly at IT site where the gap was superior.

Although the changes in efficiency of stabilizing C in the systems were noticeable in a short time scale (60 d) through the use isotopic techniques, these results could not be necessarily expected to increase the whole soil C content at the same time scale. That is because the use efficiency of the newly added ^{14}C-labelled substrate by microorganisms represents its response to microbial-derived ^{14}C formation from a constrained environment without further disturbances (i.e., no changes on soil temperature, nutrient, water, gases, microorganisms, management) whilst we do not know the extent of these disturbances over the reuse of the microbial-derived ^{14}C on the CUE. However, after more than eight years of intensification in grain production systems the changes in TN content were followed by CUE which led us to believe that the same may occur to TOC along time. Another line of evidence supporting further TOC increments is the increasing of DIN after incubation that supports the link between inorganic nutrients increase and C sequestration in annually cropped soils (Kirkby et al., 2016).

The increasing of CUE in subsoil treatments were the most consistent of the results: regardless of the system, substrate, application, or nutrient enrichment the subsoils were more efficient on stabilizing C by microorganisms than in topsoil. The results in Fig. 2 showed that subsoils potential on increasing CUE were not limited by

their relatively low microbial biomass and the decomposition pattern in Fig. 11 suggested even a decreasing in subsoil CUE after nutrient enrichment at IT site. However, we believe that subsoils were highly conducive to storing efficiently C due to their abundance of unsaturated mineral sorption surfaces linked to C-saturation model, as proved by Souza et al. (2017) studying Oxisols of wide range of texture. Also, the lower contents of TOC in subsoil treatments sustain such mechanism of C protection in depth.

4.4.2 CUE affected by substrates, applications, and nutrient enrichment

In general, the decomposition patterns of each substrate were reproducible within soil layer regardless of systems and site. Both glucose and glucosamine substrates presented initial lag-phases in subsoil treatments and so were fitted in SDM in which fundamentally relates its first phase of decay partially to microbial growth (Fig. 5 and 7). However, the microbial biomass C results could not entirely explain lag-phases in subsoil because there were not reproducible differences between layers (Fig. 2). Some studies have pointed the lag-phase as a consequence of nutrient constraining that slows the microbial C use due to SOM mining for N, P or S suiting (Creamer et al., 2016; Heitkötter et al., 2017; Liang et al., 2019). But, although low nutrient availability in subsoils and ES topsoils were detected (Table 1) the latter did not present lag-phase either because the dissolved organic forms of C and N were not considered limiting in both layers (Fig. 2). Other studies have detected initial lag-phases in decomposition of labile substrates during subsoil incubation assays which they attributed its presence and duration to dormant microorganisms community (Blagodatskaya and Kuzyakov, 2013; Placella et al., 2012). We do accept that bringing a clear evidence from glucose reapplication result in which was detected a shifting on decomposition kinetics in subsoils from SDM to EDM (Fig. 11). So, our subsoils samples could be considered predominantly colonized by dormant microorganisms but an important reservoir of biodiversity and potentially active as organic substrates turn up (Heitkötter and Marschner, 2018; Joergensen and Wichern, 2018; Kuzyakov and Blagodatskaya, 2015), as the $^{14}CO_2$ evolved from subsoils samples were on average 75.3% and 68.3% of the$^{14}CO_2$ evolved from topsoils for glucose and glucosamine respectively.

Conversely, the cellulose decay pattern presented no detectable lag-phase in subsoils (Fig. 6) which not necessarily neglected our microbial physiological evidence but even contribute to it. According to Blagodatskaya and Kuzyakov (2013), the lag-

phases are shorter for microorganisms in active physiological status thus the exponential decomposition pattern of cellulose by microorganisms in subsoils came from active and specific microbial community (K-strategists species). A large number of studies have been shown the effects of abiotic and biotic factors on soil microbial community like low nutrient availability (e.g., C, N, P, S), harsh environmental conditions (e.g. CO_2, O_2, temperature, moisture), and competition (Goberna et al., 2014; Rillig et al., 2019). These stresses cause a *in situ* pressure resulting in different functional capacities in the microbial community (Delgado-Baquerizo et al., 2016). In subsoils, limiting factors like nutrient constraints, low O_2 diffusion and lasting moisture contrast to that usually found in topsoils and so deriving specific conditions towards to competitive strategies of microorganisms for non-labile C use (Liang et al., 2019). Aligning the microbial community and physiology status, even non-dominant species can have a strong influence on framing the dominant species in a microbial community due to their underlying actions that enable the coexistence between important key species (Stolpovsky et al., 2016). This explains why the initial lag-phase occurred only at readily useful substrates and not for cellulose in subsoils. We therefore propose that the subsoil lag-phase can be explained on the recognition of soil microbial community distribution along soil profile associated to their physiological status during C supply moments, in other words, the proportion of r-strategists and k-strategist and their activity status would control the substrates decomposition in top- and subsoils.

According to Six et al., 2002, the microbial-derived substrates could be stabilized in soil through protection mechanisms against further microbial decomposition. The mechanisms involve chemical, physical and biochemical interactions between the soil and the C-substrate, such chemical stabilization through organo-mineral interactions (Castellano et al., 2015), physical protection through C occlusion in the interior of aggregates (Darrouzet-Nardi and Weintraub, 2014) and biochemical residence through molecular recalcitrance or immobilization process (Lützow et al., 2006). To associate the efficiency on stabilizing substrate-C by microorganism through the mechanisms above mentioned we considered that the ^{14}C recovery by soil extraction performed in the end of the incubations covered the releasing of substrate-derived ^{14}C remaining by organo-mineral exchanges and aggregates breakup (data not showed) (Jones and Willett, 2006; Ros et al., 2009). In our study, the average recovery of substrate-derived ^{14}C in soil extracts were 5.1% and 3.6% for glucose and cellulose meaning that the remaining still immobilized into microbial biomass, insoluble or

undertaken to some degree of recalcitrance in soil. Note that we have named the ^{14}C recovered as substrate-derived because the extractions were performed after 60 days of incubation while glucose at high concentration (i.e. 10 mM) is totally taken up by microbial biomass before 12 h (Hill et al., 2008) and cellulose can be enzymatic hydrolysed (e.g. β-cellobiosidase, β-glucosidase) to glucose at maximum rate of 30 mg C kg^{-1} in the first day of incubation (Fontaine et al., 2004) thus the ^{14}C recovered could not be considered as the initial amended substrate. On the other hand, subsoil extracts from glucosamine presented 20.5% of the total ^{14}C applied, five-fold higher than in topsoil, suggesting a more intense transformation of glucosamine through microbial biomass resulting in insoluble or recalcitrant substrate-derived ^{14}C.

Traditionally, the priming effect studies use to present the $^{14}CO_2$ evolution as rate (μg^{-1} C g^{-1} soil h^{-1}) in which some parameters are extracted for statistical analysis, like the total of substrate-derived ^{14}C mineralized (integrated curve) and the peak of the curve or the highest rate. However, this approach request that all sample must be replicated with one of them is the control and no disturbance is further applied on it (Dalenberg and Jager, 1989; Hamer and Marschner, 2005). As our purpose was robust by interacting up to five factors (system, soil layer, substrate, application, and nutrient enrichment), the priming on glucose decomposition after reapplication was showed in a different approached in this study (Fig. 9). Basically, the treatments received the ^{14}C-labelled glucose on the beginning of the incubation (1st application) followed the unlabelled glucose reapplication at day 32 (2nd application) and two decay models were fitted within each application (Fig. 1 upper). By that, from each soil sample were extracted two decay models in which the first could be used to predict the glucose decomposition as control in parallel with the priming model. The decomposition of most natural polymers releases monomeric sugars into the soil, so priming effect induces that by enhancing that sugars back to the soil (Kuzyakov, 2010). Our approach allowed us to isolate and measure the ^{14}C amount under priming decomposition (monomeric sugars) by extracting the fast pool size from the decay model that was considered consistently reproducible rather than obtaining the peak of the curve as an isolated rate. Another advantage of approaching the priming effect as $^{14}CO_2$ evolution was the possibility to calculate the CUE from the model parameters, that decreased significatively after the unlabelled glucose application (Fig. 10 upper).

An important insight was taken by decoupling the $^{14}CO_2$ measurement from the first and the second glucose applications. The glucose-^{14}C reapplication changed the

decomposition pattern in subsoils at all from SDM to EDM that was followed closely by nutrient enrichment (Fig. 11). Known that soil incubation assays have been a technique to ultimately measure the microbial activity (Dilly, 2006) and that the lag-phase can be considered a microbial growth phase (Creamer et al., 2016) we suggests that the our subsoil samples were most limited for labile-C sources . Despite that, the intensification of the microbial activity through reapplication of C and nutrients promoted loss of C so decreasing the CUE of subsoils (Fig. 10 middle). Therefore, we believe that successive pulses of C and nutrients in subsoil would lead the CUE to decrease over time, so leading its decay pattern towards that found in topsoil.

Another important result was obtained from the $^{14}CO_2$ evolved in the 2nd application at all (^{14}C-labelled and unlabelled at 2nd application) (Fig. 12). Since modelling the single application of ^{14}C-labelled glucose traces only the fate of the glucose-derived ^{14}C remaining into the soil and that is primordial to organic matter formation, modelling of successive introduction of ^{14}C-labelled glucose traces the fate of the that organic matter stabilization at all, in other words, to model the successive introduction of ^{14}C must represent a more factual dynamics of the ^{14}C into the soil organic matter (SOM). A similar approach was done by Farrar et al. (2012) that incubated ^{14}C-SOM and detected up to 99.9% of CUE. In our view, the priming through newly ^{14}C addition would be responsible for incorporating the previous ^{14}C towards a more intense action of the protection mechanisms so pushing the C to a higher degree of complexity into SOM and at same time increasing the C use efficiency by the soil.

4.4.3 Overview about metabolic process

Researchers have been modelling the kinetic transformation of soil C through single or combined linear and non-linear reactions (e.g., exponential, sigmoidal and Monod). However, the use of certain model in an attempt to estimate meaningful biological parameters and predict substrate-C decay is related to the data scatter in which depends on the variables of the soil studied, such as mineralogy, clay content, nutrient availability and microbial activity (Creamer et al., 2016, 2014; Saggar et al., 1996), but also on the external conditions like temperature and moisture (Farrar et al., 2012). In fact, even knowing the soil background and controlling the environmental/incubation conditions the decay pattern of C will also depends on the substrate type and content (Farrar et al., 2012; Hill et al., 2008). As long as we understand, under a non-limiting conditions of labile-C soil (i.e. rhizosphere) the

substrate consumption on *fast* pool derives from catabolic (i.e. maintenance-derived respiration) and anabolic process (i.e. cell growth) (Parton et al., 2015) in which their proportion will largely be responsible for driving the substrate decay pattern. That is also the matter when some labile-C substrates are applied in the soil at rates of 100 to 1000 times higher (mg C g^{-1} soil) than naturally present in soil solution (µg C g^{-1} soil), as in this study. In such cases both processes are coupled and modelling them have been the most suitable alternative for distinction (Brunner and Focht, 1984; Gillis and Price, 2011).

Our study reached a wide combination of contrasting soil treatments under a non-limiting condition of labile-C in which reflected at two distinct decay patterns of substrate-derived ^{14}C remaining in soil during the incubation that could be fitted: i) in a properly first-order reaction of exponential decay, or ii) in a first-order reaction predominately of sigmoidal shape. Since the *fast* pool (P_1) represents the size at integrated form from anabolic and catabolic processes combined together in both models (but at different proportions) they could be related each other. That was not case for the decay constants (k_1) because they derive from the differential form of the model in which k_1 was the maximum decay rate at specific time T and therefore not necessarily representing both metabolic processes (Parton et al., 2015).

The identification and splitting of metabolic processes come with a rather complex model composed by parameters related on their respective pools and decay/growth constants, which basically means that the number of parameters in the model is increased (Brunner and Focht, 1984; Scow et al., 1986). That projection brings up an increasing in the probability to overlap parameters information, that can be detected by: i) increased SEM; ii) lack of significance into model ($p > 0.100$), and iii) increased dependency value. Furthermore, the model overfitting is another reason why using the r^2 to explain the fit in the model is not suitable since the more parameter better the coefficient. In fact, we tested the fit of mixed-order models proposed by Brunner and Focht (1984) but none other complexes models were able to extract by itself the parameters from catabolic and anabolic process at all because they depend on bringing enough data density covering incubation time. Also, such complex model tests have shown us the relevance of having a proper density and range of data under studies covering a wide variation of soil treatments in which a single robust model could be fitted. That showed a limitation in our study although to predict the frequency and duration of measurements is a task made upon assumptions and based in previous

studies but also depends on resources availability (i.e., financial, time). Finally, we have not found studies approaching the CUE at deeper layers than topsoil in Oxisol and luckily our results could drive further studies looking at this topic.

4.5 Conclusions

Our study assessed the dynamics of the soil microbial community under five different factors that influence the C storage and their use efficiency by microorganisms (i.e., systems, soil layers, substrate types, applications, and nutrient enrichment). Accordingly, the most findings were:

- The difference in C use efficiency in agrosystems was consequence of the management practices adopted meaning that upgrading the intensity level in agrosystems led to decrease the C use efficiency by microorganisms.

- Higher C use efficiency was detected in subsoils due to the predominantly dormant microbial community that also was responsible for initial lag-phases in fast pool.

- Increasing the gradient of size/complexity molecule (i.e., glucose, glucosamine, and cellulose) was related to the increasing on C use efficiency by microorganisms.

- Glucose reapplication was responsible for shifting the decomposition pattern in subsoils towards that found in topsoils so decreasing the C use efficiency by microorganisms. The priming effect widely occurred after glucose reapplication in all soil samples and was related to the fast poll decay (~2%).

- Nutrient constraining in ecosystems and subsoils agrosystems did not seem to promote the initial lag-phases during decomposition kinetics. However, nutrient enrichment enhanced the decomposition rate of the fast pool during glucose reapplication compared to those without nutrients addition. A decreasing on C use efficiency was detected only at IT site.

Ultimately, the results indicated that if we aim to increase the C stock on soil, we must focus on spots with higher C use efficiency as subsoils of non-intensified agrosystems by applying techniques that allow us to reach that zone (e.g., deep-root grasses).

References

Abdalla, M., Hastings, A., Chadwick, D.R., Jones, D.L., Evans, C.D., Jones, M.B., Rees, R.M., Smith, P., 2018. Critical review of the impacts of grazing intensity on soil organic carbon storage and other soil quality indicators in extensively managed grasslands. Agric. Ecosyst. Environ. 253, 62–81. https://doi.org/10.1016/j.agee.2017.10.023

Achat, D.L., Augusto, L., Gallet-Budynek, A., Loustau, D., 2016. Future challenges in coupled C–N–P cycle models for terrestrial ecosystems under global change: a review. Biogeochemistry 131, 173–202. https://doi.org/10.1007/s10533-016-0274-9

Alvares, C.A., Stape, J.L., Sentelhas, P.C., De Moraes Gonçalves, J.L., Sparovek, G., 2013. Köppen's climate classification map for Brazil. Meteorol. Zeitschrift 22, 711–728. https://doi.org/10.1127/0941-2948/2013/0507

Amado, T.J.C., Bayer, C., Conceição, P.C., Spagnollo, E., de Campos, B.-H.C., da Veiga, M., 2006. Potential of carbon accumulation in no-till soils with intensive use and cover crops in Southern Brazil. J. Environ. Qual. 35, 1599. https://doi.org/10.2134/jeq2005.0233

Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. Eur. J. Soil Sci. 47, 151–163. https://doi.org/DOI 10.1111/j.1365-2389.1996.tb01386.x

Batlle-Bayer, L., Batjes, N.H., Bindraban, P.S., 2010. Changes in organic carbon stocks upon land use conversion in the Brazilian Cerrado: A review. Agric. Ecosyst. Environ. 137, 47–58. https://doi.org/10.1016/j.agee.2010.02.003

Baveye, P.C., Berthelin, J., Tessier, D., Lemaire, G., 2018. The "4 per 1000" initiative: A credibility issue for the soil science community? Geoderma 309, 118–123. https://doi.org/10.1016/j.geoderma.2017.05.005

Blagodatskaya, E., Kuzyakov, Y., 2013. Active microorganisms in soil: Critical review of estimation criteria and approaches. Soil Biol. Biochem. 67, 192–211. https://doi.org/10.1016/j.soilbio.2013.08.024

Blagodatsky, S., Blagodatskaya, E., Yuyukina, T., Kuzyakov, Y., 2010. Model of apparent and real priming effects: Linking microbial activity with soil organic matter decomposition. Soil Biol. Biochem. 42, 1275–1283. https://doi.org/10.1016/j.soilbio.2010.04.005

Boddey, R.M., Jantalia, C.P., Conceiçao, P.C., Zanatta, J.A., Bayer, C., Mielniczuk, J., Dieckow, J., Dos Santos, H.P., Denardin, J.E., Aita, C., Giacomini, S.J., Alves, B.J.R., Urquiaga, S., 2010. Carbon accumulation at depth in Ferralsols under zero-till subtropical agriculture. Glob. Chang. Biol. 16, 784–795. https://doi.org/10.1111/j.1365-2486.2009.02020.x

Boddy, E., Hill, P.W., Farrar, J., Jones, D.L., 2007. Fast turnover of low molecular weight components of the dissolved organic carbon pool of temperate grassland field soils. Soil Biol. Biochem. 39, 827–835. https://doi.org/10.1016/j.soilbio.2006.09.030

Brunner, W., Focht, D.D., 1984. Deterministic three-half-order kinetic model for microbial degradation of added carbon substrates in soil. Appl. Environ. Microbiol. 47, 167–172. https://doi.org/10.1128/aem.47.1.167-172.1984

Buyanovsky, G.A., Wagner, G.H., 1998. Carbon cycling in cultivated land and its global significance. Glob. Chang. Biol. 4, 131–141. https://doi.org/10.1046/j.1365-2486.1998.00130.x

Calegari, A., Hargrove, W.L., Rheinheimer, D.D.S., Ralisch, R., Tessier, D., De Tourdonnet, S., Guimarães, M.D.F., 2008. Impact of long-term no-tillage and cropping system management on soil organic carbon in an oxisol: A model for sustainability. Agron. J. 100, 1013–1019. https://doi.org/10.2134/agronj2007.0121

Castellano, M.J., Mueller, K.E., Olk, D.C., Sawyer, J.E., Six, J., 2015. Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. Glob. Chang. Biol. 21, 3200–3209. https://doi.org/10.1111/gcb.12982

Chung, H., Ngo, K.J., Plante, A., Six, J., 2010. Evidence for carbon saturation in a highly structured and organic-matter-rich soil. Soil Sci. Soc. Am. J. 74, 130. https://doi.org/10.2136/sssaj2009.0097

Corbeels, M., Scopel, E., Cardoso, A., Bernoux, M., Douzet, J.M., Neto, M.S., 2006. Soil carbon storage potential of direct seeding mulch-based cropping systems in the Cerrados of Brazil. Glob. Chang. Biol. 12, 1773–1787. https://doi.org/10.1111/j.1365-2486.2006.01233.x

Creamer, C.A., Jones, D.L., Baldock, J.A., Farrell, M., 2014. Stoichiometric controls upon low molecular weight carbon decomposition. Soil Biol. Biochem. 79, 50–56. https://doi.org/10.1016/j.soilbio.2014.08.019

Creamer, C.A., Jones, D.L., Baldock, J.A., Rui, Y., Murphy, D. V., Hoyle, F.C., Farrell, M., 2016. Is the fate of glucose-derived carbon more strongly driven by nutrient availability, soil texture, or microbial biomass size? Soil Biol. Biochem. 103, 201–212. https://doi.org/10.1016/j.soilbio.2016.08.025

Dalenberg, J.W., Jager, G., 1989. Priming effect of some organic additions to 14C-labelled soil. Soil Biol. Biochem. 21, 443–448. https://doi.org/10.1016/0038-0717(89)90157-0

Darrouzet-Nardi, A., Weintraub, M.N., 2014. Evidence for spatially inaccessible labile N from a comparison of soil core extractions and soil pore water lysimetry. Soil Biol. Biochem. 73, 22–32. https://doi.org/10.1016/j.soilbio.2014.02.010

de Vries, W., 2018. Soil carbon 4 per mille: a good initiative but let’s manage not only the soil but also the expectations: Comment on Minasny et al. (2017) Geoderma 292: 59–86. Geoderma 309, 111–112. https://doi.org/10.1016/j.geoderma.2017.05.023

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Berdugo, M., Campbell, C.D., Singh, B.K., 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nat. Commun. 7, 1–8. https://doi.org/10.1038/ncomms10541

Dilly, O., 2006. Soil Microbial Activity, in: Microbiological Methods for Assessing Soil Quality. pp. 114–182. https://doi.org/10.1079/9780851990989.0000

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x

Fabrizzi, K.P., Rice, C.W., Amado, T.J.C., Fiorin, J., Barbagelata, P., Melchiori, R., 2009. Protection of soil organic C and N in temperate and tropical soils: Effect of native and agroecosystems. Biogeochemistry 92, 129–143. https://doi.org/10.1007/s10533-008-9261-0

Farrar, J., Boddy, E., Hill, P.W., Jones, D.L., 2012. Discrete functional pools of soil organic matter in a UK grassland soil are differentially affected by temperature and priming. Soil Biol. Biochem. 49, 52–60. https://doi.org/10.1016/j.soilbio.2012.02.018

Fontaine, S., Bardoux, G., Benest, D., Verdier, B., Mariotti, A., Abbadie, L., 2004. Mechanisms of the Priming Effect in a Savannah Soil Amended with Cellulose. Soil Sci. Soc. Am. J. 68, 125–131. https://doi.org/10.2136/sssaj2004.1250

Gillis, J.D., Price, G.W., 2011. Comparison of a novel model to three conventional models describing carbon mineralization from soil amended with organic residues. Geoderma 160, 304–310. https://doi.org/10.1016/j.geoderma.2010.09.025

Glanville, H.C., Hill, P.W., Schnepf, A., Oburger, E., Jones, D.L., 2016. Combined use of empirical data and mathematical modelling to better estimate the microbial turnover of isotopically labelled carbon substrates in soil. Soil Biol. Biochem. 94, 154–168. https://doi.org/10.1016/j.soilbio.2015.11.016

Goberna, M., Navarro-Cano, J.A., Valiente-Banuet, A., García, C., Verdú, M., 2014. Abiotic stress tolerance and competition-related traits underlie phylogenetic clustering in soil bacterial communities. Ecol. Lett. 17, 1191–1201. https://doi.org/10.1111/ele.12341

Gulde, S., Chung, H., Amelung, W., Chang, C., Six, J., 2008. Soil carbon saturation controls labile and stable carbon pool dynamics. Soil Sci. Soc. Am. J. 72, 605. https://doi.org/10.2136/sssaj2007.0251

Gunina, A., Kuzyakov, Y., 2015. Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate. Soil Biol. Biochem. 90, 87–100. https://doi.org/10.1016/j.soilbio.2015.07.021

Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta analysis. Glob. Chang. Biol. 8, 345–360. https://doi.org/10.1046/j.1354-1013.2002.00486.x

Hamer, U., Marschner, B., 2005. Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. Soil Biol. Biochem. 37, 445–454. https://doi.org/10.1016/j.soilbio.2004.07.037

Haney, R.L., Franzluebbers, A.J., Porter, E.B., Hons, F.M., Zuberer, D.A., 2004. Soil carbon and nitrogen mineralization: influence of drying temperature. Soil Sci. Soc. Am. J. 68, 489–492. https://doi.org/10.2136/sssaj2004.4890

Harpole, W.S., Ngai, J.T., Cleland, E.E., Seabloom, E.W., Borer, E.T., Bracken, M.E.S., Elser, J.J., Gruner, D.S., Hillebrand, H., Shurin, J.B., Smith, J.E., 2011. Nutrient co-limitation of primary producer communities. Ecol. Lett. 14, 852–862. https://doi.org/10.1111/j.1461-0248.2011.01651.x

Heitkötter, J., Heinze, S., Marschner, B., 2017. Relevance of substrate quality and nutrients for microbial C-turnover in top- and subsoil of a Dystric Cambisol. Geoderma 302, 89–99. https://doi.org/10.1016/j.geoderma.2017.04.029

Heitkötter, J., Marschner, B., 2018. Soil zymography as a powerful tool for exploring hotspots and substrate limitation in undisturbed subsoil. Soil Biol. Biochem. 124, 210–217. https://doi.org/10.1016/j.soilbio.2018.06.021

Hessen, D.O., Ågren, G.I., Anderson, T.R., Elser, J.J., De Ruiter, P.C., 2004. Carbon sequestration in ecosystems: The role of stoichiometry. Ecology 85, 1179–1192. https://doi.org/10.1890/02-0251

Hill, P.W., Farrar, J.F., Jones, D.L., 2008. Decoupling of microbial glucose uptake and mineralization in soil. Soil Biol. Biochem. 40, 616–624. https://doi.org/10.1016/j.soilbio.2007.09.008

Hobbie, J.E., Hobbie, E.A., 2013. Microbes in nature are limited by carbon and energy: The starving-survival lifestyle in soil and consequences for estimating microbial rates. Front. Microbiol. 4, 1–11. https://doi.org/10.3389/fmicb.2013.00324

Joergensen, R.G., 2018. Amino sugars as specific indices for fungal and bacterial residues in soil. Biol. Fertil. Soils 54, 559–568. https://doi.org/10.1007/s00374-018-1288-3

Joergensen, R.G., Wichern, F., 2018. Alive and kicking: Why dormant soil microorganisms matter. Soil Biol. Biochem. 116, 419–430. https://doi.org/10.1016/j.soilbio.2017.10.022

Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem. 38, 991–999. https://doi.org/10.1016/j.soilbio.2005.08.012

Kautz, T., Amelung, W., Ewert, F., Gaiser, T., Horn, R., Jahn, R., Javaux, M., Kemna, A., Kuzyakov, Y., Munch, J.C., Pätzold, S., Peth, S., Scherer, H.W., Schloter, M., Schneider, H., Vanderborght, J., Vetterlein, D., Walter, A., Wiesenberg, G.L.B., Köpke, U., 2013. Nutrient acquisition from arable subsoils in temperate climates: A review. Soil Biol. Biochem. 57, 1003–1022. https://doi.org/10.1016/j.soilbio.2012.09.014

Khan, S.A., Mulvaney, R.L., Ellsworth, T.R., Boast, C.W., 2007. The myth of nitrogen fertilization for soil carbon sequestration. J. Environ. Qual. 36, 1821–1832. https://doi.org/10.2134/jeq2007.0099

Kirkby, C.A., Richardson, A.E., Wade, L.J., Batten, G.D., Blanchard, C., Kirkegaard, J.A., 2013. Carbon-nutrient stoichiometry to increase soil carbon sequestration. Soil Biol. Biochem. 60, 77–86. https://doi.org/10.1016/j.soilbio.2013.01.011

Kirkby, C.A., Richardson, A.E., Wade, L.J., Conyers, M., Kirkegaard, J.A., 2016. Inorganic nutrients increase humification efficiency and C-sequestration in an annually cropped soil. PLoS One 11, e0153698. https://doi.org/10.1371/journal.pone.0153698

Kirkby, C.A., Richardson, A.E., Wade, L.J., Passioura, J.B., Batten, G.D., Blanchard, C., Kirkegaard, J.A., 2014. Nutrient availability limits carbon sequestration in arable soils. Soil Biol. Biochem. 68, 402–409. https://doi.org/10.1016/j.soilbio.2013.09.032

Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol. Biochem. 34, 139–162. https://doi.org/10.1016/j.soilbio.2016.08.011

Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. Soil Biol. Biochem. 42, 1363–1371. https://doi.org/10.1016/j.soilbio.2010.04.003

Kuzyakov, Y., 2006. Sources of CO2 efflux from soil and review of partitioning methods. Soil Biol. Biochem. 38, 425–448. https://doi.org/10.1016/j.soilbio.2005.08.020

Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: Concept & review. Soil Biol. Biochem. 83, 184–199. https://doi.org/10.1016/j.soilbio.2015.01.025

Liang, Z., Olesen, J.E., Jensen, J.L., Elsgaard, L., 2019. Nutrient availability affects carbon turnover and microbial physiology differently in topsoil and subsoil under a temperate grassland. Geoderma 336, 22–30. https://doi.org/10.1016/j.geoderma.2018.08.021

Lützow, M. V., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions - A review. Eur. J. Soil Sci. 57, 426–445. https://doi.org/10.1111/j.1365-2389.2006.00809.x

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.C., Vågen, T.G., van Wesemael, B., Winowiecki, L., 2017. Soil carbon 4 per mille. Geoderma 292, 59–86. https://doi.org/10.1016/j.geoderma.2017.01.002

Mira, A.B., 2020. Carbon and nitrogen pools in the soil profile after seven years of nitrogen management and cover crop rotation in an Oxisol from Southern-Brazil.

Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide Biol. Chem. 5, 62–71. https://doi.org/10.1006/niox.2000.0319

Motavalli, P.P., Palm, C.A., Parton, W.J., Elliott, E.T., Frey, S.D., 1994. Comparison of laboratory and modeling simulation methods for estimating soil carbon pools in tropical forest soils. Soil Biol. Biochem. 26, 935–944. https://doi.org/10.1016/0038-0717(94)90106-6

Mulvaney, R.L., 1996. Nitrogen - Inorganic Forms, in: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H. (Eds.), Methods of Soil Analysis Part 3 - Chemical Methods. Soil Science Society of America, American Society of Agronomy, Madison, pp. 1123–1184. https://doi.org/10.2136/sssabookser5.3.c38

Mulvaney, R.L., Khan, S.A., Ellsworth, T.R., 2009. Synthetic nitrogen fertilizers deplete soil nitrogen: a global dilemma for sustainable cereal production. J. Environ. Qual. 38, 2295–2314. https://doi.org/10.2134/jeq2008.0527

Parton, W.J., Del Grosso, S.J., Plante, A.F., Adair, E.C., Lutz, S.M., 2015. Modeling the Dynamics of Soil Organic Matter and Nutrient Cycling, in: Soil Microbiology, Ecology, and Biochemistry. pp. 505–538. https://doi.org/10.1111/j.1365-2389.2009.01052_2.x

Paul, E.A., Paustian, K.H., Elliott, E.T., Cole, C. V., 1996. Soil organic matter in temperate agroecosystems: Long-term experiments in North America.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2018. nlme: Linear and Nonlinear Mixed Effects Models.

Placella, S.A., Brodie, E.L., Firestone, M.K., 2012. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. Proc. Natl. Acad. Sci. U. S. A. 109, 10931–10936. https://doi.org/10.1073/pnas.1204306109

Qiao, Y., Wang, J., Liang, G., Du, Z., Zhou, J., Zhu, C., Huang, K., Zhou, X., Luo, Y., Yan, L., Xia, J., 2019. Global variation of soil microbial carbon-use efficiency in relation to growth temperature and substrate supply. Sci. Rep. 9, 1–8. https://doi.org/10.1038/s41598-019-42145-6

Rillig, M.C., Ryo, M., Lehmann, A., Aguilar-Trigueros, C.A., Buchert, S., Wulf, A., Iwasaki, A., Roy, J., Yang, G., 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity. Science (80-.). 366, 886–890. https://doi.org/10.1126/science.aay2832

Roberts, P., Jones, D.L., 2012. Microbial and plant uptake of free amino sugars in grassland soils. Soil Biol. Biochem. 49, 139–149. https://doi.org/10.1016/j.soilbio.2012.02.014

Ros, G.H., Hoffland, E., van Kessel, C., Temminghoff, E.J.M., 2009. Extractable and dissolved soil organic nitrogen - A quantitative assessment. Soil Biol. Biochem. 41, 1029–1039. https://doi.org/10.1016/j.soilbio.2009.01.011

Saggar, S., Parshotam, A., Sparling, G.P., Feltham, C.W., Hart, P.B.S., 1996. 14C-labelled ryegrass turnover and residence times in soils varying in clay content and mineralogy. Soil Biol. Biochem. 28, 1677–1686. https://doi.org/10.1016/S0038-0717(96)00250-7

Sanderman, J., Hengl, T., Fiske, G.J., 2017. Soil carbon debt of 12,000 years of human land use. Proc. Natl. Acad. Sci. 114, 9575–9580. https://doi.org/10.1073/pnas.1706103114

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591–602. https://doi.org/10.1890/03-8024

Scow, K.M., Simkins, S., Alexander, M., 1986. Kinetics of mineralization of organic compounds at low concentrations in soil. Appl. Environ. Microbiol. 51, 1028–1035.

Sinsabaugh, R.L., Follstad Shah, J.J., 2012. Ecoenzymatic Stoichiometry and Ecological Theory. Annu. Rev. Ecol. Evol. Syst. 43, 313–343. https://doi.org/10.1146/annurev-ecolsys-071112-124414

Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., 2013. Carbon use efficiency of microbial communities: Stoichiometry, methodology and modelling. Ecol. Lett. 16, 930–939. https://doi.org/10.1111/ele.12113

Sisti, C.P.J., Dos Santos, H.P., Kohhann, R., Alves, B.J.R., Urquiaga, S., Boddey, R.M., 2004. Change in carbon and nitrogen stocks in soil under 13 years of conventional or zero tillage in southern Brazil. Soil Tillage Res. 76, 39–58. https://doi.org/10.1016/j.still.2003.08.007

Six, J., Conant, R.T., Paul, E. a, Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturatin of soils. Plant Soil 241, 155–176. https://doi.org/10.1023/A:1016125726789

Soil Survey Staff, 2014. Keys to soil taxonomy, Soil Conservation Service.

Souza, I.F., Almeida, L.F.J., Jesus, G.L., Kleber, M., Silva, I.R., 2017. The mechanisms of organic carbon protection and dynamics of C-saturation in Oxisols vary with particle-size distribution. Eur. J. Soil Sci. 68, 726–739. https://doi.org/10.1111/ejss.12463

Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2007. Soil carbon saturation: Concept, evidence and evaluation. Biogeochemistry 86, 19–31. https://doi.org/10.1007/s10533-007-9140-0

Stockmann, U., Padarian, J., McBratney, A., Minasny, B., de Brogniez, D., Montanarella, L., Hong, S.Y., Rawlins, B.G., Field, D.J., 2015. Global soil organic carbon assessment. Glob. Food Sec. 6, 9–16. https://doi.org/10.1016/j.gfs.2015.07.001

Stolpovsky, K., Fetzer, I., Van Cappellen, P., Thullner, M., 2016. Influence of dormancy on microbial competition under intermittent substrate supply: Insights from model simulations. FEMS Microbiol. Ecol. 92, 1–10. https://doi.org/10.1093/femsec/fiw071

Toal, M.E., Yeomans, C., Killham, K., Meharg, A.A., 2000. A review of rhizosphere carbon flow modelling. Plant Soil 222, 263–281. https://doi.org/10.1023/A:1004736021965

Van Hees, P.A.W., Jones, D.L., Finlay, R., Godbold, D.L., Lundström, U.S., 2005. The carbon we do not see - The impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: A review. Soil Biol. Biochem. 37, 1–13. https://doi.org/10.1016/j.soilbio.2004.06.010

Van Veen, J.A., Paul, E.A., 1981. Organic carbon dynamics in grassland soils. 1. Background information and computer simulation. Can. J. Soil Sci. 61, 185–201.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring microbial biomass C. Soil Biol. Biochem. 19, 703–707.

Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea: how can it occurs? Biogeochemistry 13, 87–115.

White, R.E., Davidson, B., Lam, S.K., Chen, D., 2018. A critique of the paper ‘Soil carbon 4 per mille’’ by Minasny et al. (2017).’ Geoderma 309, 115–117. https://doi.org/10.1016/j.geoderma.2017.05.025

5 FINAL CONSIDERATIONS

In Cerrado, long-term field experiments are scarce compared to many existing in the South region. So, it was preferred to go deep into a promising experiment by addressing three soil functions (i.e., soil structure, stock, and nutrient cycling) commonly studied under the effects of agricultural practice management (i.e., no-till, crop succession/rotation, N fertilization).

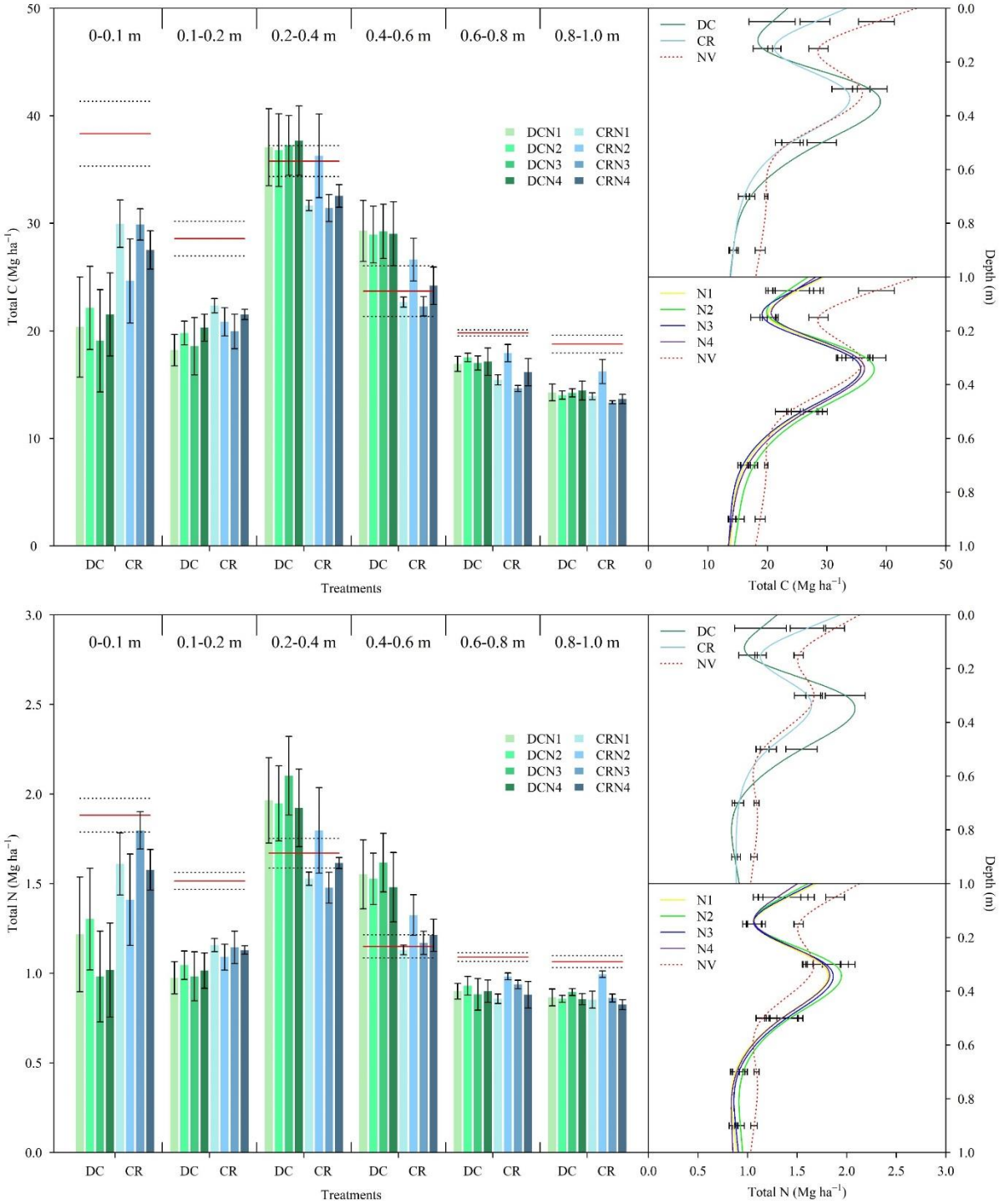
Conversely to expected, pushing the agrosystem by increasing the crop diversification did not improve the soil physical quality. This was related to the increased machine traffic needed for agrosystem intensification. Such response is a very current issue in Cerrado farms since SMPS are widely adopted for +20 years and this study showed that the soil physical quality can be affected earlier (nine years). On the other hand, the soybean-maize succession demonstrated to be quite convenient in Cerrado. It provides two harvests per year while maintaining the soil C and N stocks at reasonable levels for Cerrado soybean and maize production systems. In practice, there is a conflict into agrosystems intensification between profitability and soil physical quality depletion, apparent not only in the field but also presented in these studies.

The Cerrado also showed to be an important region to potentially increase C stocks more efficiently, mainly in the subsoil as it is a conservative spot for C stabilization. Surprisingly, the results showed that the low nutrients availability in Oxisol showed limited effects on C use efficiency and that the substrate reapplication (priming) stimulated soil microorganisms, mainly dormant in the subsoil. In practical terms, there is a great potential for C stabilization and increasing in Oxisols under Cerrado agrosystems.

Overall, although these studies were conducted independently their results were aligned. First, the conversion from native vegetation to SMPS was depletory to the assessed soil functions at all. Second, the effects of the distinct agricultural managements to soil functions evaluated herein converge allowing to hypothesize that cropping species of aggressive and deep root system (i.e., grasses) for extended period (i.e., +2 seasons) may attached the Cerrado agrosystems by improving the soil physical quality, increasing the nutrients stocks, and enhancing the CUE. Ultimately, this experiment contributed to the Cerrado grain production system and can serves as an example for others to be installed and conducted over long-term.

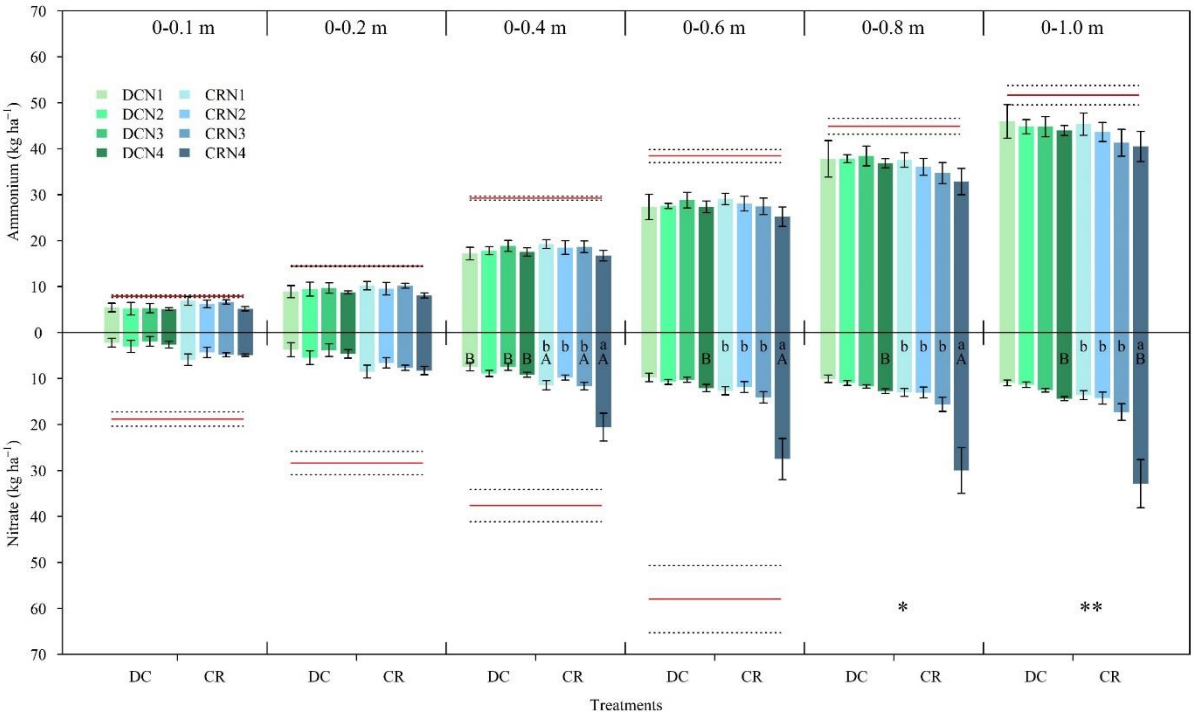
APPENDIX

Supplementary results from Section 3.

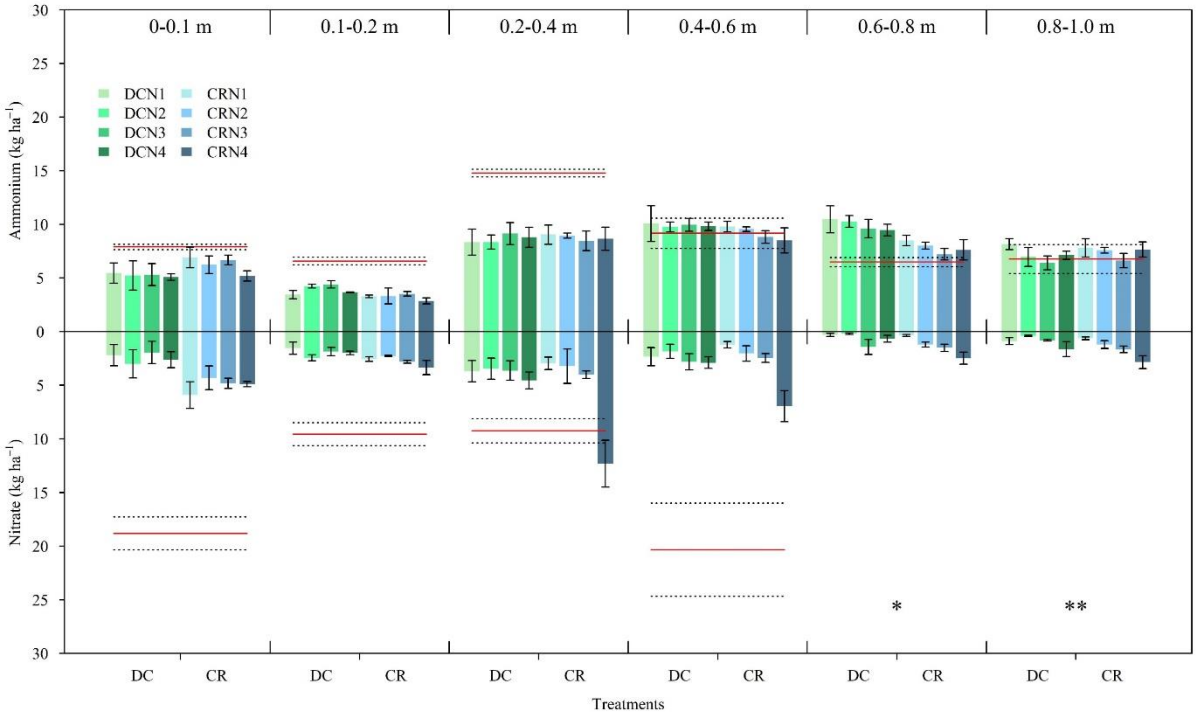


Suppl. Fig. 1. Total C (TC) and total N (TN) stocks in underlying soil layers as affected by treatments (left), SMPS (top right) and N fertilizer levels (bottom right) (without statistical test). DC: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; N1: no N application; N2: lower N level; N3: moderate N level; N4 higher N level; NV: native vegetation. Columns and

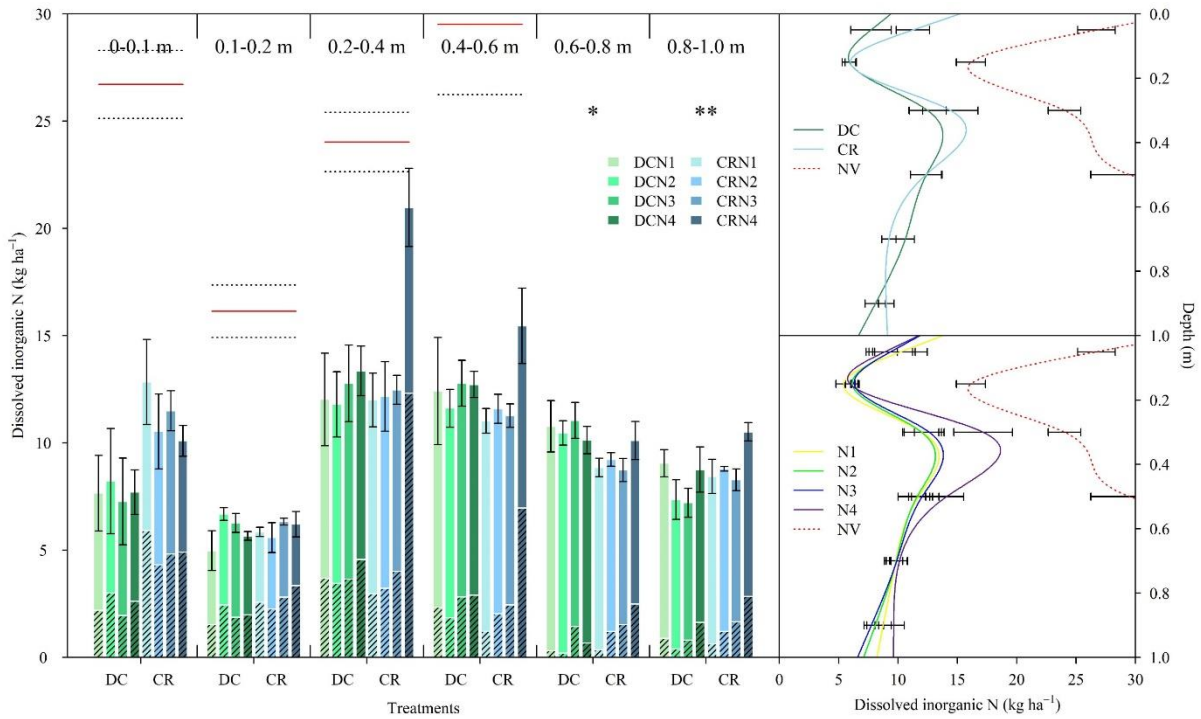
error bars represent the mean ± SEM. Red-solid lines and black-dashed lines indicate the mean ± SEM of the NV.



Suppl. Fig. 2. Ammonium and nitrate stocks accumulated in soil depth as affected by treatments. DC: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; N1: no N application; N2: lower N level; N3: moderate N level; N4 higher N level; NV: native vegetation. Columns and error bars represent the mean ± SEM. Red-solid lines and black-dashed lines indicate the mean ± SEM of the NV. * and ** respectively represent the NV mean ± SEM of nitrate stocks of 108.2 ± 10.0 and 180.8 ± 12.1 Mg ha^{-1}. Lowercase and capital letters into each column respectively indicate differences among treatments at N fertilizer level inside SMPS and at SMPS inside N fertilizer levels, according to Tukey's test (p < 0.100).



Suppl. Fig. 3. Ammonium and nitrate stocks in underlying soil layers as affected by treatments (without statistical test). DC: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; N1: no N application; N2: lower N level; N3: moderate N level; N4 higher N level; NV: native vegetation. Columns and error bars represent the mean ± SEM. Red-solid lines and black-dashed lines indicate the mean ± SEM of the NV. * and ** respectively represent the NV mean ± SEM of nitrate stocks of 50.2 ± 4.0 and 72.6 ± 6.4 Mg ha^{-1}.



Suppl. Fig. 4. Dissolved inorganic forms of N stocks in underlying soil layers as affected by treatments (without statistical test). DC: soybean/maize succession under no-till; CR: crop rotation under no-till including soybean, maize, grasses, and legumes in rotation; N1: no N application; N2: lower N level; N3: moderate N level; N4 higher N level; NV: native vegetation. Columns and error bars represent the mean ± SEM of DIN and dashed columns the mean of nitrate. Red-solid lines and black-dashed lines indicate the mean ± SEM of the NV. * and ** respectively represent the NV mean ± SEM of DIN stocks of 56.7 ± 3.6 and 79.4 ± 5.2 Mg ha^{-1}.

Supplementary results from Section 4.

Suppl. Table 2. Effect of system and soil layer on parameter values for triple EDM (topsoil) and SDM (subsoil) describing the decomposition of ^{14}C-labelled glucose added as shown in Fig. 2.

Treat.	$P_{1.3}$ (%)	$k_{1.3(s)}$ (d^{-1})	T (d)	$P_{2.3}$ (%)	$k_{2.3}$ (d^{-1})	$P_{3.3}$ (%)
Topsoil						
SA_{IT}	44.9 ± 3.7 bA***	1.53 ± 0.39 ab***	-	13.7 ± 2.2 B***	0.079 ± 0.012 bA*	44.5 ± 1.3 aB***
IA_{IT}	47.7 ± 1.3 abA***	1.83 ± 0.07 a***	-	15.2 ± 0.8 B***	0.109 ± 0.003 aA***	40.2 ± 0.9 abB***
ES_{IT}	55.0 ± 0.6 aA***	0.66 ± 0.03 b***	-	13.6 ± 0.4 B***	0.063 ± 0.005 bA*	35.6 ± 0.9 bB***
Subsoil						
SA_{IT}	25.9 ± 4.4 B***	-0.46 ± 0.08 **	9.9 ± 0.5 ***	20.0 ± 2.2 abA***	0.031 ± 0.006 B*	55.3 ± 2.3 bA***
IA_{IT}	24.9 ± 3.1 B*	-0.38 ± 0.09 *	11.6 ± 1.5 ***	25.1 ± 3.6 aA***	0.029 ± 0.006 B*	51.1 ± 1.6 bA***
ES_{IT}	17.0 ± 1.0 B***	-0.52 ± 0.01 ***	10.5 ± 0.2 ***	18.3 ± 0.6 bA***	0.033 ± 0.001 B***	65.2 ± 1.6 aA***
Topsoil						
SA_{PG}	54.2 ± 0.5 aA***	1.25 ± 0.02 b***	-	15.8 ± 0.5 bB***	0.075 ± 0.007 A***	33.3 ± 0.9 bB***
IA_{PG}	55.2 ± 0.8 aA***	1.18 ± 0.03 b***	-	14.3 ± 0.2 bB***	0.061 ± 0.002 ***	33.8 ± 0.6 bB***
ES_{PG}	38.3 ± 2.7 bA***	1.76 ± 0.22 a***	-	24.6 ± 3.1 aB***	0.078 ± 0.011 A***	39.0 ± 0.4 a***
Subsoil						
SA_{PG}	23.6 ± 0.6 B***	-0.71 ± 0.03 **	7.0 ± 1.0 ***	31.5 ± 0.8 bA***	0.051 ± 0.003 B***	45.2 ± 0.5 aA***
IA_{PG}	25.2 ± 1.5 B***	-0.81 ± 0.04 ***	6.0 ± 0.5 ***	30.0 ± 0.9 bA***	0.056 ± 0.001 ***	45.0 ± 0.8 aA***
ES_{PG}	25.6 ± 2.4 B***	-0.82 ± 0.05 ***	8.1 ± 0.3 ***	35.5 ± 2.2 aA***	0.052 ± 0.002 B***	39.0 ± 2,1 b***

IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. $P_{1.3}$: fast pool size (% ^{14}C-labelled glucose), $k_{1.3(s)}$: EDM ($k_{1.3}$) or SDM ($k_{1.3s}$) growth/decay rate at fast pool (d^{-1}), T: inflection point where $k_{1.3s}$ is maximum and $P_{1.3}$ is half for fast pool at SDM, $P_{2.3}$: slow pool size (% ^{14}C-labelled glucose), $k_{2.3}$: decay rate at slow pool (d^{-1}), $P_{3.3}$: passive pool size (% ^{14}C-labelled glucose). $P_{3.3}$ had no decay cause its constant had no significance for up to 60-days incubation period (commonly $p > 0.500$) and so could be denominated as asymptote at triple EDM and SDM. Values represent means ± SEM (n = 4) of the measured replicates. For interactions split into sites ($P_{1.3}$, $P_{2.3}$, $k_{2.3}$, $P_{3.3}$), lowercase letters indicate significant differences among systems within soil layers while uppercase indicate significant differences among soil layers within systems. For main effects split into sites ($k_{1.3(s)}$, T) lowercase letters indicate significant differences among systems (without interactions). Note SDM decay of the inverted sigmoidal function on subsoil implies on negative values at $k_{1.3s}$. Superscripts signals indicates the significance level of the parameter for the model fit: *** < 0.001, ** < 0.010, * < 0.050, $^{'}$ < 0.100.

Suppl. Table 3. Effect of system and soil layer on parameter values for triple EDM describing the decomposition of ^{14}C-labelled cellulose added as shown in Fig. 3.

Treat.	$P_{1.3}$ (%)	$k_{1.3}$ (d^{-1})	$P_{2.3}$ (%)	$k_{2.3}$ (d^{-1})	$P_{3.3}$ (%)
Topsoil					
SA_{IT}	13.5 ± 1.4 A***	0.62 ± 0.07 B***	11.0 ± 0.6 bA*	0.023 ± 0.005 bB	75.4 ± 1.1 aB***
IA_{IT}	15.7 ± 0.9 A***	0.77 ± 0.03 B***	14.7 ± 0.7 aA***	0.058 ± 0.006 aA***	69.4 ± 0.4 bB***
ES_{IT}	14.9 ± 0.6 A***	0.42 ± 0.03 ***	15.5 ± 0.4 aA***	0.042 ± 0.005 a***	70,1 ± 1.1 bB***
Subsoil					
SA_{IT}	0.3 ± 0.1 bB$^{'}$	12.89 ± 3.92 aA$^{'}$	8.2 ± 0.6 B***	0.045 ± 0.004 A***	91.6 ± 0.5 aA***
IA_{IT}	0.4 ± 0.1 abB$^{'}$	6.46 ± 2.16 bA$^{'}$	8.6 ± 1.2 B***	0.045 ± 0.003 B***	91.0 ± 1.3 aA***
ES_{IT}	2.7 ± 0.4 aB***	0.44 ± 0.01 b**	9.6 ± 0.2 B***	0.034 ± 0.001 ***	87.7 ± 0.6 bA***
Topsoil					
SA_{PG}	18.0 ± 1.0 aA***	0.76 ± 0.05 A***	14.7 ± 0.2 bA***	0.053 ± 0.005 ***	66.9 ± 1.1 aB***
IA_{PG}	14.7 ± 0.5 bA***	0.72 ± 0.02 A***	20.1 ± 1.6 aA***	0.054 ± 0.007 ***	64.8 ± 1.2 abB***
ES_{PG}	16.2 ± 0.8 abA***	0.82 ± 0.13 A***	19.4 ± 0.6 aA***	0.055 ± 0.003 A***	63.7 ± 0.6 bB***
Subsoil					
SA_{PG}	4.4 ± 0.3 B***	0.51 ± 0.03 B***	10.0 ± 0.9 B***	0.046 ± 0.006 ***	85.7 ± 0.9 A***
IA_{PG}	4.8 ± 0.4 B***	0.57 ± 0.07 B***	8.6 ± 0.4 B***	0.049 ± 0.003 ***	86.7 ± 0.4 A***
ES_{PG}	6.2 ± 0.5 B***	0.44 ± 0.03 B***	9.9 ± 0.5 B***	0.039 ± 0.002 B**	84.0 ± 1.0 A***

IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. $P_{1.3}$: fast pool size (% ^{14}C-labelled glucose), $k_{1.3}$: decay rate at fast pool (d^{-1}), $P_{2.3}$: slow pool size (% ^{14}C-labelled glucose), $k_{2.3}$: decay rate at slow pool (d^{-1}), $P_{3.3}$: passive pool size (% ^{14}C-labelled glucose). $P_{3.3}$ had no decay cause its constant had no significance for up to 60-days incubation period (commonly $p > 0.500$) and so could be denominated as asymptote at triple EDM. Values represent means ± SEM (n = 4) of the measured replicates. For interactions split into sites ($P_{1.3}$, $k_{1.3}$, $P_{2.3}$, $k_{2.3}$, $P_{3.3}$), lowercase letters indicate significant differences among systems within soil layers while uppercase indicate significant differences among soil layers within systems. Superscripts signals indicates the significance level of the parameter for the model fit: *** < 0.001, ** < 0.010, * < 0.050, $^{'}$ < 0.100.

Suppl. Table 4. Effect of system and soil layer on parameter values for double EDM (topsoil) and SDM (subsoil) describing the decomposition of ^{14}C-labelled glucosamine added as shown in Fig. 4.

Treat.	$P_{1.2}$ (%)	$k_{1.2(s)}$ (d^{-1})	T (d)	$P_{2.2}$ (%)	$k_{2.2}$ (d^{-1})
Topsoil					
SA_{IT}	42.3 ± 0.5 bA***	0.47 ± 0.05 ***	-	59.3 ± 0.6 aB***	0.0014 ± 0.0001 a***
IA_{IT}	43.2 ± 0.7 ab***	0.46 ± 0.01 ***	-	58.5 ± 0.7 ab***	0.0012 ± 0.0001 a***
ES_{IT}	47.5 ± 0.6 a***	0.39 ± 0.05 ***	-	55.0 ± 0.5 b***	0.0008 ± 0.0001 b$^{'}$
Subsoil					
SA_{IT}	25.1 ± 2.5 cB***	-0.05 ± 0.01 b***	33.2 ± 2.2 ab***	78.2 ± 2.5 aA***	-
IA_{IT}	41.4 ± 0.7 b***	-0.10 ± 0.01 a***	29.1 ± 0.1 b***	60.1 ± 0.1 b***	-
ES_{IT}	46.5 ± 2.7 a***	-0.07 ± 0.01 b***	37.5 ± 1.9 a***	57.1 ± 2.1 b***	-
Topsoil					
SA_{PG}	55.4 ± 0.6 A***	0.35 ± 0.02 b***	-	46.8 ± 0.4 B***	0.0018 ± 0.0002 b**
IA_{PG}	57.0 ± 0.6 A***	0.43 ± 0.02 b***	-	46.0 ± 0.5 B***	0.0019 ± 0.0002 b**
ES_{PG}	52.6 ± 0.6 B***	0.55 ± 0.06 a***	-	50.1 ± 0.7 ***	0.0035 ± 0.0002 a***
Subsoil					
SA_{PG}	46.5 ± 2.9 bB***	-0.07 ± 0.01 ***	29.8 ± 0.2 b***	57.9 ± 3.2 aA***	-
IA_{PG}	49.5 ± 2.5 bB***	-0.07 ± 0.01 ***	35.6 ± 0.9 a***	53.5 ± 2.7 aA***	-
ES_{PG}	58.3 ± 1.0 aA***	-0.10 ± 0.02 ***	27.2 ± 2.2 b***	45.9 ± 0.8 b***	-

IT: Itiquira site; PG: Ponta Grossa site; SA: standard agrosystem; IA: intensified agrosystem; ES: ecosystem. $P_{1.2}$: fast pool size (% ^{14}C-labelled glucose), $k_{1.2(s)}$: EDM ($k_{1.3}$) or SDM ($k_{1.3s}$) growth/decay rate at fast pool (d^{-1}), T: inflection point where $k_{1.2s}$ is maximum and $P_{2.3}$ is half for fast pool at SDM, $P_{2.2}$: slow + passive pool size (% ^{14}C-labelled glucose), $k_{2.2}$: decay rate at slow + passive pool (d^{-1}). $P_{2.2}$ at subsoil treatments had no decay cause its constant had no significance for up to 60-days incubation period (commonly $p > 0.500$) and so could be denominated as asymptote at double EDM and SDM. Values represent means ± SEM (n = 4) of the measured replicates. For interactions split into sites ($P_{1.2}$, $P_{2.2}$), lowercase letters indicate significant differences among systems within soil layers while uppercase indicate significant differences among soil layers within systems. For main effects split into sites ($k_{1.2(s)}$, T, $k_{1.2}$) lowercase letters indicate significant differences among systems (without interactions). Note SDM decay of the inverted sigmoidal function on subsoil implies on negative values at $k_{1.3s}$. Superscripts signals indicates the significance level of the parameter for the model fit: *** < 0.001, ** < 0.010, * < 0.050, $^{'}$ < 0.100.