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COMUNIDADE MICROBIANA DO SOLO E ATIVIDADE ENZIMÁTICA EM UM LATOSSOLO SUBTROPICAL SOB PLANTIO DIRETO DE LONGA DURAÇÃO E ROTAÇÃO DE CULTURAS

Santa Maria, RS
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Dissertação apresentada ao Curso de Pós-Graduação em Ciência do Solo, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciência do Solo**.

Orientador: Prof. Dr. Telmo Jorge Carneiro Amado

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Aprovado em 27 de abril de 2018:

Prof. Dr. Telmo Jorge Carneiro Amado (UFSM)
(Presidente/Orientador)

Prof. Dr. Celso Aita (UFSM)

Prof. Dr. Charles W. Rice (Kansas State University - USA)
(Videoconferência)

Santa Maria, RS
2018

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“Seja você quem for, seja qual for a posição social que você tenha na vida, a mais alta ou a mais baixa, tenha sempre como meta muita força, muita determinação e sempre faça tudo com muito amor e com muita fé em Deus, que um dia você chega lá. De alguma maneira você chega lá”

(Ayrton Senna da Silva)

RESUMO

COMUNIDADE MICROBIANA DO SOLO E ATIVIDADE ENZIMÁTICA EM UM LATOSSOLO SUBTROPICAL SOB PLANTIO DIRETO DE LONGA DURAÇÃO E ROTAÇÃO DE CULTURAS

AUTOR: Carlos Augusto Bonini Pires
ORIENTADOR: Telmo Jorge Carneiro Amado

O desenvolvimento e a seleção de práticas agrícolas tem sido um dos principais desafios para a sustentabilidade dos agroecossistemas frente ao aumento da demanda por alimentos. Nesse sentido, a utilização de sistemas conservacionistas, caracterizados pelo mínimo distúrbio do solo (sistema de plantio direto – SPD), permanente cobertura do solo e rotação de culturas, pode promover uma melhoria na comunidade microbiana do solo, em comparação com solos intensivamente revolvidos. Entretanto, nas principais regiões agrícolas da América do Sul, o SPD tem sido frequentemente utilizado em desconjunto dos demais princípios dos sistemas conservacionistas, deliberando extensas áreas de monocultura. Portanto, é possível esperar que a associação do SPD e o aumento da diversidade de culturas agrícolas empregadas no sistema de rotação, venha a beneficiar a comunidade microbiana e atividade enzimática do solo. Nesse sentido, foi hipotetizado que o SPD associado a uma rotação de culturas frequente e diversificada pode beneficiar a composição e a atividade microbiana do solo. O experimento foi implantado em 1985 (32 anos) em um Latossolo Vermelho Distrófico típico em Cruz Alta – RS. O clima do local é subtropical úmido (Cfa) com uma precipitação média anual de 1774 mm e temperatura média de 25ºC. Os tratamentos foram constituídos por dois sistemas de manejo de solo (SPD e plantio convencional) e três sistemas de rotação de culturas. Amostras de solo foram coletadas em três profundidades: 0 a 5, 5 a 10 e 10 a 30 cm. A comunidade microbiana do solo foi acessada através dos ácidos graxos fosfolipídicos (PLFA). A atividade das enzimas β-glucosidase, acid phosphatase e N-acetyl-glucosaminidase (biomarcadores da ciclagem do carbono, fósforo e nitrogênio, respectivamente), foi determinada através de fluorometria. A maior biomassa microbiana, soma de todos os biomarcadores PLFA, foi reportada na camada de 0 a 5 cm m sob SPD (40,19 nmol PLFA g^{-1} solo), enquanto no plantio convencional, na mesma camada, a biomassa microbiana foi de 25,41 nmol PLFA g^{-1} solo. Por outro lado, a biomassa microbiana do solo foi aumentada em profundidade (10 a 30 cm) pelo plantio convencional. Esse resultado pode ser suportado pelo incremento de carbono e nutrientes como Ca^{2+} e Mg^{2+} e, o declínio do teor de Al^{3+}, como resultado da retenção de resíduos de plantas e nutrientes na superfície do solo para o SPD, e da incorporação desses resíduos e nutrientes para o manejo convencional do solo. Além disso, a associação do SPD com o sistema de rotação de culturas com maior diversidade de plantas, incrementou a atividade de todas as enzimas testadas na camada de 0 a 5 cm. Entretanto, a diferença entre os sistemas de rotação de culturas decresceu com a profundidade do solo e foi discreta sob plantio convencional. Os resultados indicam que o aumento da diversidade das plantas empregadas nos sistemas de rotações de culturas favorece a abundância da comunidade microbiana e a atividade das enzimas sob SPD.

Palavras-Chave: Biomassa microbiana do solo. Ácidos graxos fosfolipídicos (PLFA). Atividade de hidrolases. Plantas de cobertura. Revolvimento do solo.

ABSTRACT

SOIL MICROBIAL COMMUNITY AND ENZYME ACTIVITY IN A SUBTROPICAL OXISOL UNDER LONG-TERM NO-TILL AND CROP ROTATIONS

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ADVISOR: Telmo Jorge Carneiro Amado

The development and selection of agricultural practices are one of the main challenges for the sustainability of agroecosystems in response to the increased demand for food. Thus, mediate agricultural practices based on conservation agriculture, characterized by minimal soil disturbance (e.g., no-till - NT), permanent soil cover, and crop rotation can promote an improvement in the soil microbial community in comparison with tilled soils. However, NT is often used without the other principles of conservation agriculture in South America. Thus, the combination of NT and increasing crop rotation diversity may enhance the soil microbial community and enzymes activity. In this way, we hypothesized that no-till adoption without crop diversification, a practice used in large scale in South America, won't be able to increase the soil microbial biomass and activity. The long-term experiment was established at the CCGL-TEC in Cruz Alta, state of Rio Grande do Sul, Southern Brazil, in a Typic Hapludox. The climate was classified as humid subtropical (Cfa) with average annual precipitation of 1774 mm and the average temperature of 25ºC. The experiment consisted of two soil management systems: no-tillage (NT) and conventional tillage (CT), and three crop rotation systems. Soil samples were taken at 0 to 5, 5 to 10, and 10 to 30 cm. The soil microbial community was accessed by phospholipid fatty acid analysis (PLFA). We used a fluorometric method to evaluate β-glucosidase, acid phosphatase, and N-acetyl-glucosaminidase activity as biomarkers of C, N and P cycling. The highest microbial biomass was reported at 0 to 5 cm layer under NT (40.19 nmol PLFA g-1 soil), whereas CT was 25.41 nmol PLFA g-1 soil. On another hand, soil microbial biomass was augmented in deeper soil layers (10 to 30 cm) of the CT soil. The higher abundance of microbial groups in the 0 to 5 cm and 10 to 30 cm layers of respective NT and CT soils were correlated with increased C and nutrient levels (N, Ca2+, Mg2+) and decreased Al3+ concentrations, as a result of plant residue and nutrient retention on the surface of NT soils and incorporation within the plow layer in CT soils. Moreover, the association of NT and increased crop rotation augmented the activity of β-glucosidase, acid phosphatase, and N-acetyl-glucosaminidase in the topsoil. Nevertheless, differences among cropping systems decreased with soil depth and were discrete under CT. Our results suggest that the increase in crop diversity favored the abundance of both microbial communities and extracellular enzymes activity in the surface of NT soils.

Keywords: Soil microbial biomass. Phospholipid fatty acid (PLFA). Hydrolase activity. Cover crops. Soil tillage.

SUMÁRIO

1 INTRODUÇÃO GERAL

Os solos desempenham um papel essencial para a vida, regulando processos através de diversos ecossistemas. Atualmente, está se explorando os solos mais produtivos do planeta, porém, a erosão do solo, perda da biodiversidade, decréscimo do teor de matéria orgânica e a depleção de nutrientes, associados a degradação, vem tornando o modelo de agricultura atual insustentável (ADEWOPO *et al.*, 2014; AMUNDSON *et al.*, 2015; KARLEN; RICE, 2015).

Melhorar as práticas agrícolas tem sido um dos principais desafios para a sustentabilidade dos agroecossistemas, principalmente na medida em que a demanda por alimentos aumenta (FOLEY *et al.*, 2011; PITTELKOW *et al.*, 2014), já que são as práticas agrícolas que medeiam os níveis de produção de alimentos (KARLEN; RICE, 2015; TILMAN *et al.*, 2011). Portanto, avançar na melhoria das práticas agrícolas para aumentar a qualidade do solo e a produção das culturas é um dos fatores chave para promover a segurança alimentar. Apesar de extensivamente discutido, ainda há uma lacuna de conhecimento sobre as práticas agrícolas capazes de promover, concomitantemente, a qualidade dos solos, a produtividade das culturas comerciais e a minimização da degradação do solo. A degradação do solo é uma preocupação recorrente em regiões subtropicais, especialmente pelo uso intensivo do solo e carência de rotação de culturas nos sistemas produtivos (FU *et al.*, 2015; TORRES-SALLAN *et al.*, 2017), causando um declínio na saúde e qualidade desses solos (BRIEDIS *et al.*, 2018; KHALEDIAN *et al.*, 2017; TUZZIN DE MORAES *et al.*, 2016).

Nesse sentido, a utilização de sistemas conservacionistas, caracterizados pelo mínimo distúrbio do solo (isto é, sistema de plantio direto – SPD), permanente cobertura do solo e rotação de culturas (PITTELKOW *et al.*, 2014; TERAVEST *et al.*, 2015) podem contribuir para suprir essas lacunas. Diversos estudos comprovam que o SPD enriquece o sistema solo, melhorando as propriedades químicas, físicas e biológicas em comparação com solos intensivamente revolvidos (BLANCO-CANQUI *et al.*, 2009; HANSEL *et al.*, 2017; REICHERT *et al.*, 2016; SIX; PAUSTIAN, 2014). Entretanto, nas principais regiões agrícolas do Brasil, o SPD tem sido frequentemente utilizado em desconjunto dos demais princípios dos sistemas conservacionistas, deliberando extensas áreas de monocultura. Agroecossistemas baseados em monocultura apresentam baixa cobertura do solo, baixa estabilidade de agregados, maior compactação do solo, menor infiltração da água no solo e, consequentemente, maior erosão e escoamento superficial (CHAVARRÍA *et al.*, 2016; REICHERT *et al.*, 2016). Em contrapartida, já é conhecido que sistemas agrícolas que

contemplam a rotação de culturas, são providos de uma maior ciclagem de nutrientes, maior estabilidade de agregados e de uma maior proteção contra os impactos das chuvas (BRIEDIS *et al.*, 2018; FERRARI *et al.*, 2015). Além disso, através do aumento da intensidade e diversidade da rotação de culturas, projeta-se que pode aumentar a produtividade agrícola e a eficiência do uso da água e nutrientes. Portanto, é possível esperar que a associação do SPD e o aumento da intensidade e diversidade da rotação de culturas venha a beneficiar a comunidade microbiana e atividade enzimática do solo (AI *et al.*, 2018; ASCHI *et al.*, 2017; FABRIZZI *et al.*, 2009; ZUBER *et al.*, 2017), conhecidos por mediar diversos ciclos biogeoquímicos no solo (WHITE; RICE, 2009).

Diversos fatores bióticos e abióticos, alterados pelo manejo do solo e rotação de culturas, regulam a composição da comunidade microbiana. Os fatores bióticos incluem predação, competição e simbiose (FAKRUDDIN; MANNAN, 2013). Os fatores abióticos incluem fatores físicos e químicos, como disponibilidade de água e nutrientes, aeração, temperatura, composição bioquímica dos resíduos culturais, pH, pesticidas e atividades humanas. Nesse sentido, por exemplo, os fungos preferem ambientes com menor pH e alta relação C:N, degradando carboidratos como a celulose. Do mesmo modo, os actinomicetos (filo de bactérias gram-positivas), se comportam de modo semelhante aos fungos, já que criam extensivos micélios e ajudam a decompor a matéria orgânica de organismos mortos. Em contrapartida, as bactérias são mais adaptadas em ambientes que disponham matéria orgânica como fonte de carbono (WAN *et al.*, 2014), já que preferem substratos mais lábeis e ricos em N. Além disso, os níveis de pH, manganês e zinco são fatores determinantes para o crescimento e desenvolvimento dos fungos micorrizicos arbusculares. Não obstante, enzimas hidrolíticas como a β-glucosidase, acid phosphatase e N-acetyl-glucosaminidase, são responsáveis por mediar a ciclagem de nutrientes como carbono, nitrogênio e fósforo (BOWLES *et al.*, 2014; ZHAO *et al.*, 2016). A atividade potencial dessas enzimas é frequentemente ligada à biomassa microbiana e usada como indicadora da demanda de carbono, nitrogênio e fósforo da comunidade microbiana.

No entanto, estudos sobre a comunidade microbiana e atividade enzimática em experimentos de longa duração com sistemas de preparo de solo e rotação de culturas distintos ainda são escassos. Nesse sentido, hipotetizou-se que o SPD associado a uma rotação de culturas frequente e diversificada pode aumentar a biomassa e a diversidade microbiana do solo. Essa hipótese foi testada acessando a comunidade microbiana e a atividade enzimática em um experimento de longa duração (32 anos) com sistemas de manejo de solo e rotação de culturas contrastantes.

2 HIPÓTESE

Sistema de plantio direto associado à rotação de culturas frequente e diversificada pode aumentar a biomassa e a diversidade microbiana do solo.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Definir práticas agrícolas que venham a contribuir no desenvolvimento da comunidade microbiana e na atividade enzimática de um Latossolo em condições subtropicais.

3.2 OBJETIVOS ESPECÍFICOS

a) Avaliar o efeito de diferentes sistemas de preparo de solo e rotação de culturas na comunidade de bactérias gram-positivas, bactérias gram-negativas, actinomicetos, fungos e fungos micorrizicos arbusculares;
b) Avaliar o efeito de diferentes sistemas de preparo de solo e rotação de culturas na atividade das enzimas hidroliticas β-glucosidase, acid phosphatase e N-acetyl-glucosaminidase.

4. ARTIGO – Soil microbial community and enzyme activity in a subtropical Oxisol under long0term no-till and crop rotations

Abstract

Soil microbial community and enzymes activity are known to regulate carbon and nutrient cycling in agricultural soils. No-till promotes soil carbon sequestration and increases carbon storage in comparison with intensively tilled soils. It is well known that the introduction of crop rotation in agricultural systems improves nutrient cycling, aggregate stability, and soil protection against the rainfall impact. However, due to the monoculture of soybean, the use of crop rotation is still marginal in agroecosystems in South America. Thus, the association of no-till with a diversified crop rotation into the cropping system may enhance the soil microbial communities to increase soil carbon and nutrient availability in comparison with tilled soils with no crop rotation. We tested this hypothesis assessing the soil microbial community and enzyme activity in a long-term (32 yr.) experiment with contrasting soil management and cropping systems in Southern Brazil. The experiment was established in 1985 on a Typic Hapludox in Cruz Alta-RS (Brazil) under no-tillage (NT) and conventional tillage (CT). Three cropping systems with increasing crop rotation diversity based on winter/summer crops were tested: wheat /soybean (R0); black oat/soybean/wheat/soybean (R1); and black oat/soybean/black oat+ common vetch/maize/radish/wheat/soybean (R2). The samples were taken at the 0 to 5 cm, 5 to 10 cm, and 10 to 30 cm. The soil microbial community was accessed by phospholipid fatty acid analysis (PLFA). We used a fluorometric method to evaluate β-glucosidase, acid phosphatase, and N-acetyl-glucosaminidase activity as biomarkers of C, N and P cycling. Long-term NT increased microbial biomass as evaluated by total PLFA biomarkers in the topsoil (0 to 5 cm). In contrast, soil

microbial communities were augmented in deeper soil layers (10 to 30 cm) of the CT soil. The association of NT and increased crop rotation augmented the activity of β-glucosidase, acid phosphatase, and N-acetyl-glucosaminidase in the topsoil. Nevertheless, differences among cropping systems decreased with soil depth and were discrete under CT. The higher abundance of microbial groups in the 0 to 5 cm and 10 to 30 cm layers of respective NT and CT soils were correlated with increased C and nutrient levels (N, Ca2+, Mg2+) and decreased Al3+ concentrations, as a result of residue and nutrient retention on the surface of NT soils and incorporation within the plow layer in CT soils. Our results suggest that increase on crop diversity favored the abundance of both microbial communities and extracellular enzymes activity in the surface of NT soils, thus supporting that reduced soil disruption and agricultural intensification may increase soil health and nutrient cycling in agroecosystems.

Keywords:

Soil microbial biomass; phospholipid fatty acid (PLFA); hydrolase activity; cover crops; soil tillage

Introduction

The development and selection of agricultural practices are one of the main challenges for the sustainability of agroecosystems in response to the increased demand for food (Foley et al., 2011; Pittelkow et al., 2014; Chi et al., 2016). Therefore, improvement of agricultural practices is a crucial factor to improve soil health and promote food security since the agricultural practices mediate food production levels (Tilman et al., 2011, 2002; Karlen and Rice, 2015). Although extensively discussed, a knowledge gap still exists for agricultural practices capable

of promoting soil quality concomitantly and provide high efficiency in the use of agricultural inputs. However, soil degradation is a recurring concern in tropical regions due to inadequate soil management practices, primarily by intensive soil tillage and the lack of crop diversification (Fu et al., 2015; Pittelkow et al., 2015; Torres-Sallan et al., 2017), resulting in a decline of microbial community, soil health, and crop yield (Khaledian et al., 2017; Moraes et al., 2016; de Oliveira Ferreira et al., 2018).

Thus, mediate agricultural practices based on conservation agriculture, characterized by minimal soil disturbance (e.g., no-till - NT), permanent soil cover, and crop rotation (Derpsch and Friedrich, 2009; Hobbs et al., 2008; Pittelkow et al., 2014; TerAvest et al., 2015), can contribute to microbial community development and soil health. No-till can improve soil chemical, physical and microbiological properties in comparison with intensively tilled soils (Blanco-Canqui et al., 2009; Six and Paustian, 2014; de Oliveira Ferreira et al., 2016; Reichert et al., 2016a, 2016b; Hansel et al., 2017). However, NT is often used without the other principles of conservation agriculture that include permanent soil covering and crop rotation (Pittelkow et al., 2014). It is well known that the introduction of crop rotation in agricultural systems promotes nutrient cycling, aggregate stability, and soil protection against the rainfall impact (Ferrari et al., 2015; Chavarría et al., 2016; Briedis et al., 2018). However, due to the monoculture of soybean, the use of crop rotation and cover crops is still marginal in agroecosystems in South America, especially in Brazil and Argentina (Wingeyer et al., 2015). Crop rotation may increase agricultural productivity, nutrient water use efficiency (Lal, 2015; Pittelkow et al., 2014; Tilman et al., 2002; Wu, 2018), and soil microbial community (Ai et al., 2018; Aschi et al., 2017; Fabrizzi et al., 2009; Ferrari et al., 2015; Rodrigues et al., 2013; Zuber et al., 2017)

by increasing crop frequency and species diversity (i.e. agricultural intensification). Thus, the combination of NT and increasing crop rotation diversity may enhance the soil microbial community and enzymes activity, known to regulate several biogeochemical cycles of carbon and nutrients (Ashworth et al., 2017; White and Rice, 2009). Nonetheless, studies on microbial community and enzyme activity in long-term experiments with contrasting soil management and cropping systems are still scarce and poorly documented. We hypothesized that no-till adoption without crop diversification, a practice used in large scale in South America, won't be able to increase the soil microbial biomass and activity. We tested this hypothesis by assessing soil microbial community and enzyme activity in a long-term (32 yr.) experiment with contrasting soil management and cropping systems in Southern Brazil.

Materials and methods

Study site and field characteristics

The long-term experiment was established at the CCGL-TEC in Cruz Alta, state of Rio Grande do Sul, Southern Brazil (28º36' S, 53º40' W, elevation: 409 m, slope: 4%), in April 1985. The climate was classified as humid subtropical (Cfa) according to Peel et al. (2007) with average annual precipitation of 1774 mm and an average temperature of 25ºC. The rainfall was evenly distributed during the year, but with droughts occurring in some years (Fig. 1). During the experiment period, the site experienced udic and ustic conditions. The soil at the site was a Typic Hapludox (Soil Survey Staff, 2014), hereafter referred to as an Oxisol, predominantly composed of kaolinite and iron oxides. Table 1 document the soil physicochemical properties means for each treatment.

Table 1. Soil physicochemical properties with depth by different soil management and cropping systems (n=3).

Tillage* Cropping System	Clay	Nitrogen	Carbon	K^+	P	pH	Al^{3+}	Ca^{2+}	Mg^{2+}	CEC_{eff}	EC
	---------- % ----------			---- mg dm^{-3} ----			---------- $cmol_c$ dm^{-3} ----------				μS cm^{-1}
						0-5 cm					
CT-R0	58.00(1.53)	0.12(4e-3)	1.67(0.04)	160.40(29.79)	15.50(6.97)	5.35(0.03)	0.34(0.02)	4.52(0.31)	3.25(0.16)	10.53(1.18)	101.53(4.22)
CT-R1	55.00(1.00)	0.13(0.01)	1.73(0.06)	89.33(22.58)	14.96(4.13)	5.22(0.10)	0.36(0.06)	4.53(0.65)	3.19(0.29)	8.31(0.92)	67.93(7.92)
CT-R2	52.67(1.20)	0.14(3e-3)	1.76(0.03)	138.8(14.88)	14.2(1.32)	5.12(0.05)	0.62(0.07)	4.46(0.37)	3.42(0.33)	8.52(0.23)	87.57(15.87)
NT-R0	47.67(2.85)	0.22(4e-3)	2.68(0.04)	105.60(28.74)	27.9(0.95)	6.14(0.31)	0.00(0.00)	5.62(0.60)	3.88(0.27)	9.76(0.94)	146.2(10.28)
NT-R1	47.33(1.76)	0.20(0.01)	2.39(0.13)	87.73(19.26)	35.2(9.32)	5.70(0.23)	0.00(0.00)	6.00(0.65)	3.72(0.31)	8.85(0.64)	165.00(3.18)
NT-R2	45.67(2.60)	0.23(0.03)	3.05(0.23)	110.13(33.71)	23.16(9.13)	5.77(0.09)	0.00(0.00)	6.15(0.89)	4.11(0.24)	9.94(0.99)	228.77(11.83)
						5-10 cm					
CT-R0	58.33(0.33)	0.12(3e-3)	1.69(0.02)	104.80(23.74)	20.79(6.22)	5.34(0.16)	0.34(0.07)	4.63(0.17)	3.29(0.19)	8.46(0.21)	77.08(3.66)
CT-R1	55.00(0.58)	0.13(0.01)	1.67(0.08)	98.27(11.09)	17.54(4.43)	5.27(0.10)	0.28(0.04)	3.98(0.42)	3.05(0.21)	7.57(0.51)	73.5(3.21)
CT-R2	52.33(0.33)	0.14(5e-3)	1.80(0.02)	67.73(6.27)	19.86(9.07)	5.23(0.04)	0.40(0.02)	4.40(0.55)	3.17(0.24)	8.53(0.21)	74.54(4.70)
NT-R0	56.33(1.45)	0.10(0.02)	1.51(0.03)	58.8(17.45)	18.24(3.72)	5.39(0.24)	0.66(0.24)	3.72(0.65)	3.05(0.26)	7.58(0.72)	72.46(5.85)
NT-R1	56.33(0.33)	0.12(4e-3)	1.58(0.05)	51.73(8.78)	17.92(5.18)	4.97(0.28)	1.38(0.99)	3.48(0.48)	2.75(0.19)	8.13(0.80)	102.55(5.99)
NT-R2	53.33(0.88)	0.13(0.01)	1.66(0.08)	54.80(17.42)	17.53(1.94)	4.97(0.09)	1.06(0.21)	4.04(0.08)	3.22(0.14)	7.74(0.35)	101.81(10.77)
						10-30 cm					
CT-R0	58.33(1.20)	0.11(0.01)	1.50(0.15)	88.27(14.08)	13.47(1.48)	5.57(0.14)	0.24(0.06)	4.82(1.01)	3.57(0.48)	7.62(0.52)	74.68(8.26)
CT-R1	56.33(0.88)	0.12(0.01)	1.58(0.12)	91.6(31.46)	17.03(4.48)	5.29(0.12)	0.26(0.09)	4.16(0.25)	3.19(0.18)	7.83(0.37)	84.44(6.70)
CT-R2	50.00(0.58)	0.11(0.01)	1.51(0.08)	72.53(36.74)	15.95(1.67)	5.36(0.07)	0.36(0.07)	4.54(0.56)	3.21(0.23)	8.85(1.40)	65.34(17.78)
NT-R0	58.33(0.67)	0.09(4e-3)	1.39(0.07)	42.40(6.47)	3.97(1.67)	4.92(0.16)	1.50(0.60)	3.17(0.96)	2.49(0.23)	7.26(0.79)	66.79(4.18)
NT-R1	55.33(0.88)	0.11(0.01)	1.53(0.07)	36.00(6.99)	5.33(2.06)	5.07(0.13)	1.44(0.82)	2.80(0.47)	2.49(0.28)	8.29(0.76)	52.21(17.78)
NT-R2	55.00(1.73)	0.10(1e-3)	1.36(0.10)	36.67(16.68)	5.92(1.18)	5.28(0.21)	0.64(0.43)	4.09(0.66)	2.79(0.27)	6.83(0.34)	59.56(8.05)

Means for physicochemical properties with the standard error of the mean in brackets.

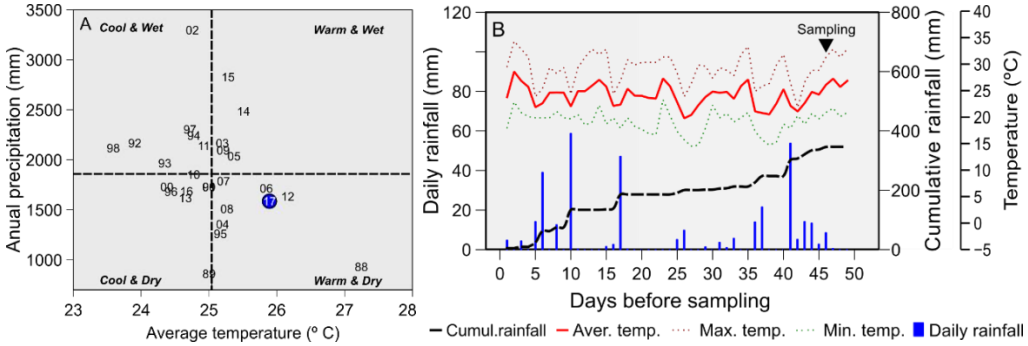

Fig. 1. a) Annual temperature and precipitation summaries for the experimental location using the historical weather years (1988 – 2017) (blue is the year of sampling). Vertical and horizontal lines show the average annual temperature and precipitation, respectively; b) Daily rainfall, cumulative rainfall and maximum, average and minimum temperature close to sampling.

The experiment was set up following a split-plot arrangement without replications. Hence, three pseudo-replications were allocated within the sub-plots. The research consisted of two soil management systems: no-tillage (NT) and conventional tillage (CT) with each plot measuring 40m wide and 60 m long (2,400m^2), allowing all field operations similar to a commercial farm. The NT was performed by sowing crops with minimal soil disturbance, and the CT consisted of disk plow (20 cm) followed by twice disk tandem (10 cm) prior to sowing the summer and winter crops. Only before radish (rotation R2), there was no soil tillage. Three cropping systems (Fig. 2), with increasing crop rotation diversification based on winter/summer crops, were tested: wheat (*Triticum aestivum* L.)/soybean (*Glycine max* L.) (R0); black oat (*Avena strigose* S.)/soybean/wheat/soybean (R1); and black oat/soybean/black oat+ common vetch (*Vicia sativa* L.)/maize (*Zea mays* L.)/radish (*Raphanus sativus* L.)/wheat/soybean (R2). Prior the establishment of the experiment in 1985, the area was managed with conventional tillage for 30 years first with wheat (monoculture) and wheat/soybean (succession winter/summer), where the wheat straw was burned. The plots were amended with lime and fertilized with N, P and K

following soil analysis (CQFS-RS/SC, 2016). Other soil and management practices follow local best management practices.

Soil sampling

Soil sampling occurred in February 2017 (Fig. 1b). The samples were taken at the 0 to 5; 5 to 10 and; 10 to 30 cm soil depth for all variables analyzed, excluding soil organic carbon (C) and total nitrogen (N) that were taken at the 0 to 5; 5 to 15 and; 15 to 30 cm soil depth. Soil samples were collected with a spatula from an open pit (90 x 90 cm) with 90 cm depth. Soil samples for microbial properties were kept in a cooler (4ºC) and frozen (-20 ºC) within 2 hrs after sampling and stored until analysis. Samples for particle size analysis and chemical properties were cleaned of roots, air-dried, ground, and sieved (2 mm) at room temperature.

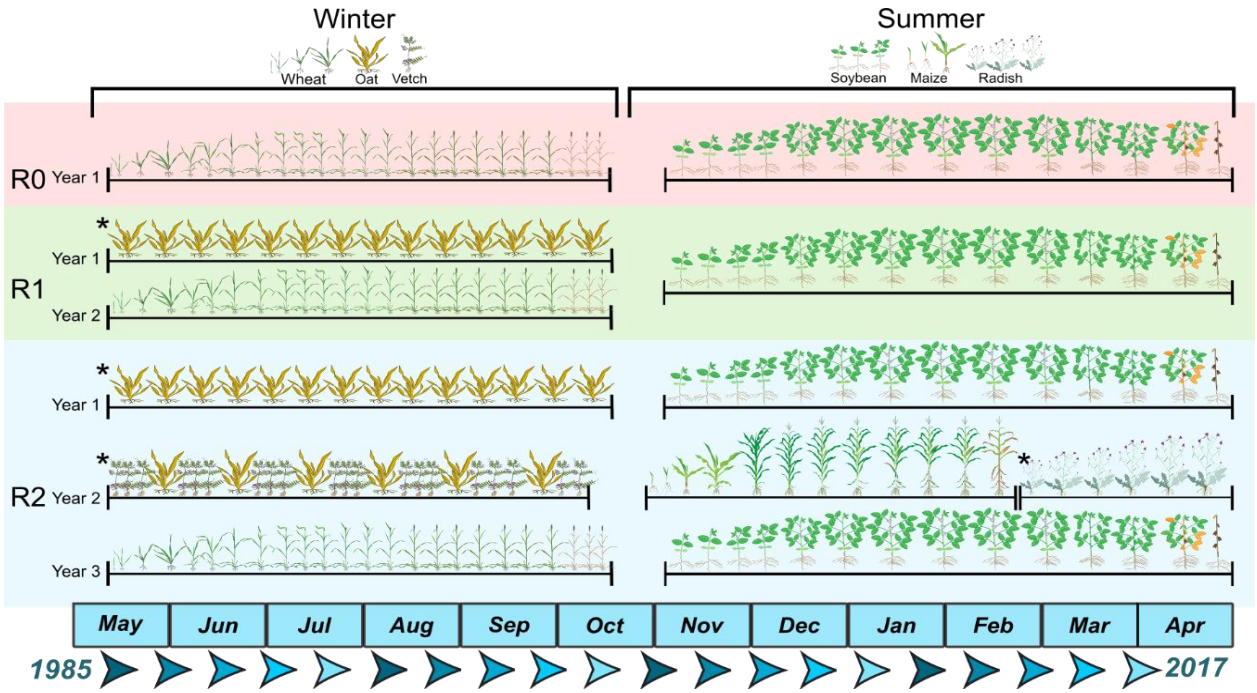

Fig. 2. Cropping systems description. All cropping systems were tested under no-tillage and conventional tillage. Cover crops were used without the purpose of harvesting. R0: 1 year to complete the rotation system; R1: 2 years to complete the rotation system; R2: 3 years to complete the rotation system.
***** Cover crops

Soil chemical properties analysis

Sub-samples were finely grounded in a ball mill and analyzed for soil organic carbon (C) and total nitrogen (N) by dry combustion using a C/N Elemental Analyzer (Flash EA 1112 Series ThermoFinnigan Italia S.p.A./ MI, Italy). Soil water pH and saturated soil-paste electrical conductivity (EC) was measured in a saturation extract (1:1 soil: water) (Embrapa, 2011). Potassium (K^+) and phosphorus (P) were extracted with a Mehlich-I solution. K content was determined by flame photometry, and the P content was measured colorimetrically using molybdenum blue (Embrapa, 2011). Calcium (Ca^{2+}), magnesium (Mg^{2+}), and aluminum (Al^{3+}) were extracted using 1.0 mol L^{-1} KCl. Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrophotometry. Al was titrated with NaOH 0.025 mol L^{-1} (Embrapa, 2011). The effective cation exchange capacity (CEC) of the soil was determined by the sum of the exchangeable bases (K^+, Ca^{2+}, and Mg^{2+}) plus Al^{3+}.

Soil microbiological properties analysis.

Phospholipid fatty acid (PLFA) analysis was performed with modifications on the original procedure (White and Ringelberg, 1998). Total lipids were extracted by 10 mL of methanol, 5 mL of chloroform, and 4 mL of phosphate buffer (pH 7.4) on 5 g freeze-dried soil. Water and chloroform were added after 3 hr to separate the mixture into polar and nonpolar fractions, while total lipids remained in the nonpolar phase. Phospholipids were isolated from neutral lipids and glycolipids by using silicic acid chromatography columns (Disposable BAKERBOND® SPE Columns, J.T. Baker®) and eluted with methanol. The phospholipids were then saponified by KOH, methylated to fatty acid methyl esters (FAME), and analyzed by Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham,

Massachusetts, USA) equipped with a DB5-MS column (30 m x 250 μm in diameter x 0.25 μm film thickness; Agilent Technologies, Santa Clara, California, USA). Helium was used as the carrier gas. FAME peaks were recognized by different retention time in comparison with the bacterial acid methyl esters mix (BAME; Matreya 1114; Matreya LLC, Pleasant Gap, Pennsylvania, USA). Internal standards 19:0 FAME were used to determine concentrations.

A total of 30 biomarkers were identified for all samples. Microbial groups were assigned based on characteristics of the biomarkers: iso and ante-iso branched often belong to gram-positive bacteria; monosaturated and cyclopropyl lipids often belong to gram-negative bacteria; actinobacteria have more methyl-branched fatty acids, and methyl linoleate typically belong to fungi (Table 2). Phospholipid fatty acid abundance was reported as nmol per gram of dry soil (nmol PLFA g^{-1} soil). Total bacteria were the sum of gram-positive bacteria, gram-negative bacteria, and actinomycetes. Total fungi were the sum of fungi and arbuscular mycorrhizal fungi. Microbial biomass (MB) was the sum of all PLFA biomarkers.

Table 2. PLFA signatures chosen to characterize microbial community structure

Microbial group	Abbreviation	Fatty acids
Gram-positive bacteria	Gram+	i15:0; a15:0; i16:0; i17:0; a17:0
Gram-negative bacteria	Gram-	cy19:0; cy17:0; 2-OH 10:0; 2-OH 12:0; 3-OH 12:0; 2-OH 14:0; 3-OH 14:0; 2-OH 16:0; 16:1ω7
Actinomycetes	Actino	10-methyl 18:0; 10-methyl 19:0
Fungi	Fungi	18:2ω9, 12
Arbuscular mycorrhizal fungi	AMF	16:1ω5

Hydrolytic enzyme assays include C-requiring enzyme (β-glucosidase, βG, EC 3.2.1.21), P-requiring enzyme (acid phosphatase, AcidP, EC 3 .1.3.2), and N-requiring enzyme (N-acetyl-glucosaminidase, NAG, EC 3.2.1.52). The potential activities of hydrolases were measured following a modified fluorometric method using fluorometric substrates 4-methylumbelliferone (Zeglin et al., 2013). One gram

of soil was homogenized in 100 mL of 50 mM pH 5 acetate buffer. Soil slurries were added into 96-well microplates with 200 µM fluorometric substrate proxy specific to each enzyme. Each sample had six analytical replicates. Additionally, buffer blank, soil blank, negative control, 4-methylumbelliferone reference standard, and quench control were measured with each sample to adjust enzyme activity value. Specific incubation time was measured for each enzyme. The time that 0.5 N NaOH solution was added in hydrolases activity assays was recorded as stop times. Fluorescent absorbance was determined by a Multi-Mode Microplate Reader (FilterMax F5, Molecular Devices, USA) with 365/450 nm excitation/emission for hydrolase fluorometric plates. Potential enzyme activities were reported as nanomoles activity per gram of dry soil per hour ($nmol^{-1}$ hr^{-1} g^{-1} soil).

Statistical analysis

The effect of soil management, cropping system, depth, and their respective interactions (fixed effects) on the response variables was analyzed by ANOVA with a mixed model. Random effects corresponded to i) cropping system within soil management [cropping system (soil management)], and ii) depth within soil management × cropping system [depth (soil management × cropping system)]. Normality of the residuals was investigated using the Shapiro-Wilk test, and square-root transformation was applied when necessary. To account for potential spatial correlation of the plotted errors, exponential, Gaussian and spherical correlation functions without nugget effect were evaluated using the *nlme* package (Pinheiro et al., 2017) of the R statistical software(R Core team, 2018). This step was added in order to overcome the lack of randomization for soil sampling inherent to this long-term experiment. Moreover, all the models were adjusted with homogenous and

heterogeneous variances for the different depths using the *weights* function from the *nlme* package. Model selection for correlation structure was done following the Akaike information Criterium (AIC) and Bayesian Information Criterium (BIC). When comparing homoscedastic and heteroscedastic models, Likelihood Ratio Test (LRT) was used (West et al., 2007). A post-hoc comparison was used to determine significant differences among treatments for all the fixed effects presenting a significance equal or lower than 0.05 using the Tukey's Honest Significant Difference test (*multicomp* R package, (Hothorn et al., 2008).

Distance-based redundancy analysis (dbRDA) (Legendre and Andersson, 1999) was used to explore linear relationships between key soil physicochemical properties and axes of the ordination of sites based upon Bray–Curtis dissimilarities utilizing the function capscale of the *vegan* package (Oksanen et al., 2017) in R software. Their significance was tested by a Monte Carlo permutation test using 999 permutations. Prior to analysis, biological data were log transformed. In the present study, dbRDA was performed separately for each of the three soil depths. The direction and magnitude of the relationship between environmental and biological variables were presented visually in the dbRDA triplot (sites as points and biological and environmental as vectors). Moreover, a Pearson correlation analysis was performed for all variables studied for the purpose of accompanying the dbRDA correlations.

Results

Changes in soil microbial community

Soil microbial communities were assessed by the abundance of PLFA biomarkers according to the soil management, cropping system, and sampling depth

(Table 3). Significant one-way and two-way interaction (*P* <0.05) were noticed, respectively, for cropping system and among soil management*depth (Appendix A). Cropping system affected all PLFA biomarkers including soil microbial biomass, which were significantly higher in the cropping systems with cover crops in the rotation (R1: 25.3% and R2: 21.6%). However, no differences were found between R1 and R2 treatments.

Table 3. Changes in soil microbial community by different soil management, cropping systems, and depths. Results are presented in nmol PLFA g^{-1} soil.

Source of variation	Microbial Biomass	Total Bacteria	Total Fungi	Gram +	Gram -	Actino**	AMF***	Fungi
Crop. system	*P*=0.01269*	*P*=0.0048	*P*=0.01506	*P*=0.00364	*P*=0.00580	*P*=0.01053	*P*=0.01247	*P*=0.0316
R0	19.97 b	8.39 b	4.38 b	4.28 b	3.19 b	0.93 b	3.24 b	1.14 b
R1	26.76 a	10.84 a	5.68 a	5.68 a	4.00 a	1.16 a	4.08 a	1.60 a
R2	25.48 a	10.64 a	5.59 a	5.40 a	4.11 a	1.14 a	3.94 a	1.66 a
Tillage*Depth	*P*=≤.0001	*P*=≤.0001	*P*=≤.0001	*P*=≤.0001	*P*=0.0001	*P*=≤.0001	*P*=≤.0001	*P*=≤.0001
CT-0_5 cm	25.41 b	10.78 b	5.62 b	5.71 b	4.00 b	1.07 b	3.93 b	1.69 b
CT-5_10 cm	24.69 b	10.47 b	5.46 b	5.46 bc	3.89 b	1.12 b	3.90 b	1.55 b
CT-10_30 cm	21.40 b	8.89 b	4.40. b	4.61 bc	3.30 b	0.98 bc	3.24 b	1.16 b
NT-0_5 cm	40.19 a	15.08 a	9.15 a	8.33 a	5.30 a	1.46 a	6.41 a	2.74 a
NT-5_10 cm	20.09 bc	8.70 b	4.46 b	4.01 c	3.63 b	1.06 b	3.30 b	1.16 b
NT-10_30 cm	12.62 c	5.83 c	2.22 c	2.58 d	2.49 c	0.76 c	1.72 c	0.49 c

means followed by the same letter are not different according to the Tukey test at the 5% level.
*Type III Tests of Fixed Effects P value
** Actinomycetes
*** Arbuscular mycorrhizal fungi

As expected, tillage*depth interaction had a significant effect on all PLFA biomarkers tested. Nonetheless, the distribution of the microbial communities among soil layers was similar for different PLFA biomarkers. The higher microbial biomass was established in the soil surface (0-5 cm) under NT (40.19 nmol PLFA g^{-1} soil), decreasing by 50% (5-10 cm) and 68.6% (10-30 cm) with an increase in soil depth. No differences in soil microbial biomass, total bacteria, total fungi, Gram+, Gram-, Actino, AMF and, fungi were found among soil layers with CT, which had a mean of 23.83 nmol PLFA g^{-1} soil for microbial biomass. Nevertheless, the microbial biomass and all other biomarkers investigated were higher at 10 to 30 cm layer of CT soil in comparison with NT, except for Actino.

Furthermore, AMF composes more than 70% of the total fungi, regardless of the treatment. In the same way, Gram+ bacteria represented 55.23%, 46.09%, and 44.25%, respectively, according to the depth increment of the total bacteria under NT. Conversely, Gram- bacteria decreased with depth, typifying 35.14%, 41.72%, and 42.71%, respectively, of the total bacteria according to the depth under NT.

Changes in extracellular enzyme activity

Significant three-way interaction (P <0.05) among soil management, cropping system, and soil depth was documented for βG activity (Appendix A). Soil βG activity decreased with depth, regardless of the soil management system, excluding NTR1 treatment (Fig. 3). The βG activity was 69% higher in the 0 to 5 cm soil layer of NT than CT. In the same layer, βG activity was enhanced by increasing crop rotation diversity in NT, but no effect was noticed in CT soil. The difference of NTR2 for NTR0 and NTR1 were, respectively, 23% and 33%.

Likewise, three-way interaction (P <0.05) among soil management, cropping system, and soil depth was documented for AcidP (Appendix A). For NAG activity, the analysis of variance revealed significant two-way interaction (P <0.05) among soil management*cropping system, soil management*depth, and cropping system*depth (Appendix A). Acid P and NAG activity (Fig. 3) was sensitive to the cropping system under NT, primarily in the soil surface. In this situation, crop rotation diversity increased the activity of these enzymes, regardless of the soil management system. The high-diversity crop rotation (R2) increased the activity of AcidP in the surface (0-5 cm) in CT. The same did not occur for NAG which had an increase at 5 to 10 cm, excluding NTR0 and NTR2. Moreover, AcidP was most affected by cropping system and NAG by soil management.

Overall, excluding NAG activity in the NTR1 and NTR2 treatments and especially for βG activity, NT system created an enzyme activity gradient. Under CT, the activity distribution in the entire soil profile was more homogeneous.

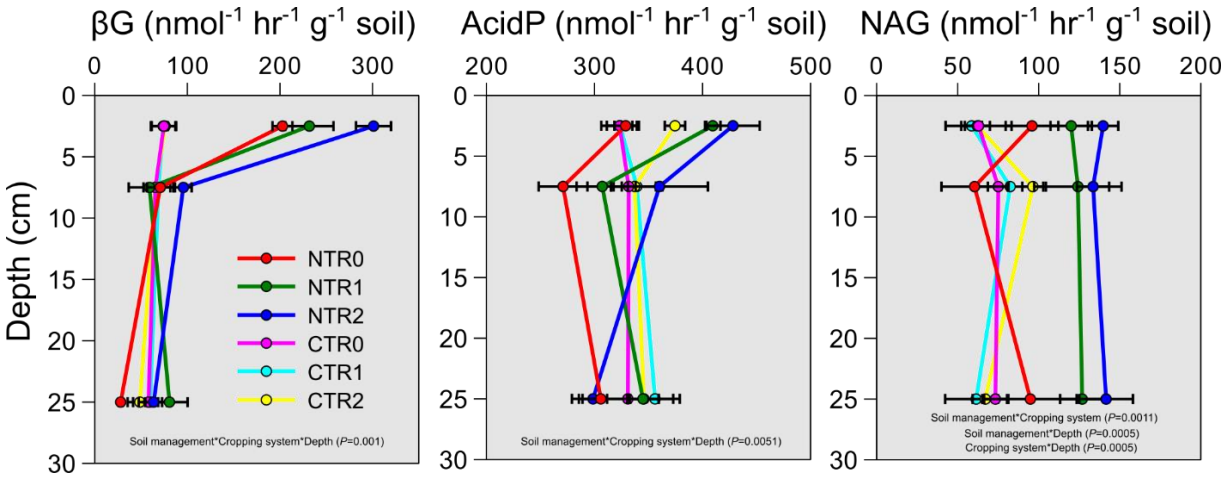

Fig. 3. Soil extracellular enzyme activities affected by different soil management, cropping systems, and depths (n = 3). Error bars represent 95% confidence intervals.

Relative influence of soil physicochemical attributes on the microbial community and enzyme activity.

To examine the relative influence of soil physicochemical properties on the microbial community and enzyme activity was performed a distance-based redundancy analysis (dbRDA for each studied soil depth.

For the 0 to 5 cm depth, dbRDA of the PLFA groups and enzymes was constrained by all soil physicochemical properties which explained 79.7% of the total variance by the first two axes (with 999 permutations, F= 7.05, P= 0.002) as influenced by soil management and cropping system (Fig. 4a). The variability of PLFA and enzymes was strongly related to pH (F= 20.74, P= 0.002), Al^{3+} (F= 17.73, P= 0.002), Ca^{2+} (F= 8.76, P= 0.013) and EC (F= 11.85, P= 0.012) (Fig. 1) as a result of the of Monte Carlo permutation test (P≤0.05). Only the first axis (CAP 1, horizontal) was significant (F= 54.46, P<0.003), which was significant and strongly

related to the aforementioned variables explaining 76.27% of the total variance. Microbial community and enzymes activities were positively correlated with C, N, Ca^{2+}, Mg^{2+}, EC, CEC, P and pH, and negatively correlated with clay and Al^{3+} (Fig. 4a). Ordination of treatments was primarily related to CAP 1 separating the CT system treatments from the NT treatments (Fig. 4a). The permutation test for dbRDA model demonstrated no significant effect (with 999 permutations, F= 0.79, P= 0.679) for 5 to 10 cm soil depth (Fig. 4b).

For the 10 to 30 cm depth, dbRDA analysis revealed that the first and second axis combined accounted for 80.16% of the total variation (with 999 permutations, F= 4.00, P= 0.01) (Fig. 2). Ordination was mainly related to CAP 1 as this axis was the only significant axis (F= 32.99, P= 0.006), explaining 73.97% of the total variance (Fig. 4c). The variability of PLFA biomarkers and enzymes was significantly related to pH (F= 5.71, P= 0.027), Al^{3+} (F= 6.18, P= 0.021), P (F= 7.77, P= 0.009), CEC (F= 5.55, P= 0.020), and EC (F= 6.77, P= 0.016) from the Monte Carlo permutation test (P≤0.05) (Fig. 2). Microbial community and enzymes activities (excluding NAG) were positively correlated with C, N, Ca^{2+}, Mg^{2+}, EC, CEC, P and pH, and negatively correlated with clay and Al^{3+} (Fig 4c).

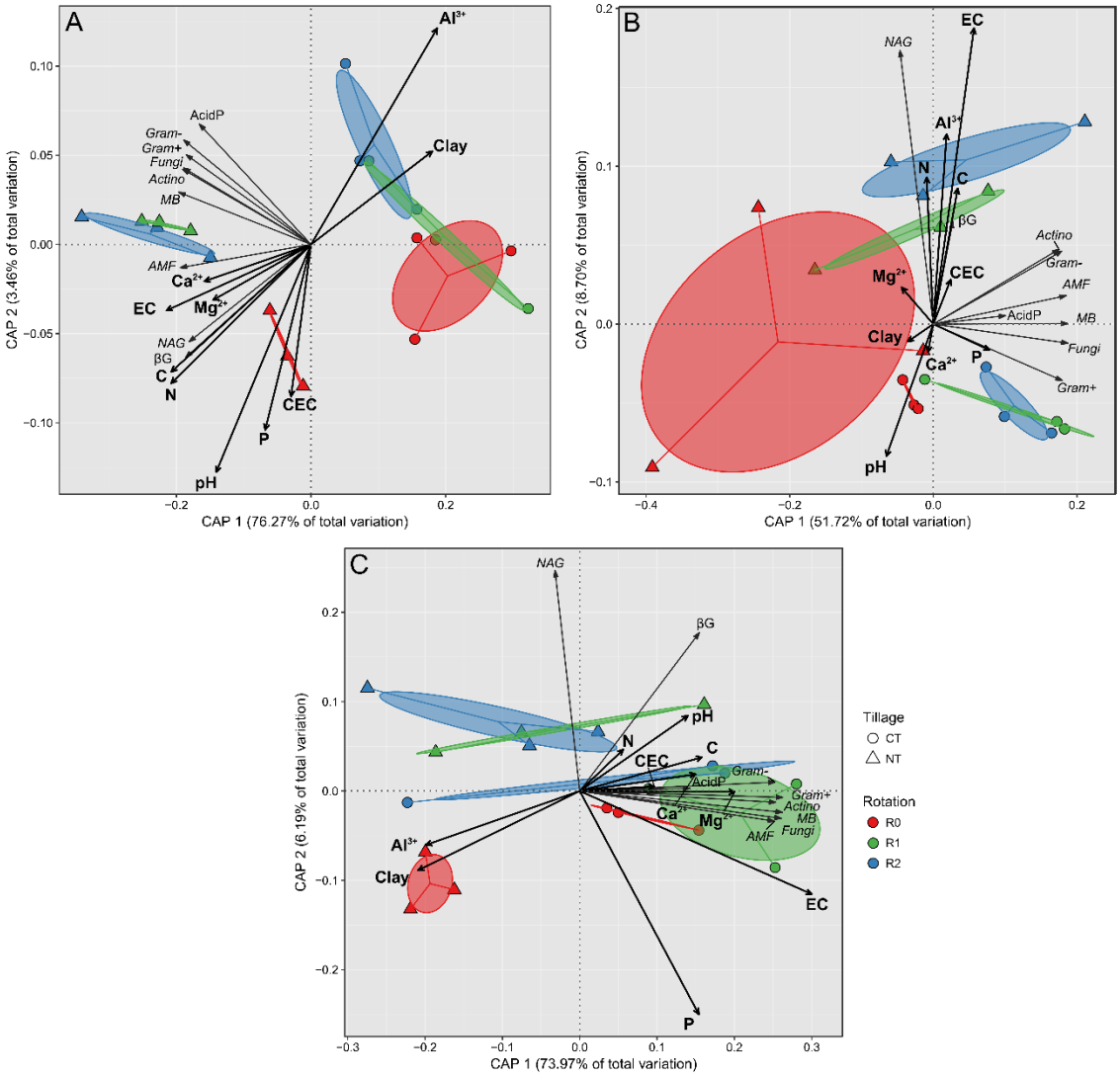

Fig. 4. Distance-based redundancy analysis (dbRDA) of microbial community and enzyme activities constrained by soil physicochemical properties at 0 to 5 cm (A), 5 to 10 cm (B), and 10 to 30c m (C).

Discussion

Changes in soil microbial community

This study provides insights on the understanding of the soil microbial community in a subtropical Oxisol under contrasting soil management and cropping systems. Cropping system significantly affected the soil microbial biomass and community. We hypothesized that crop rotation diversity could affect positively microbial community composition and this hypothesis was accepted. Different cover crops and cash crops (that's is, plant species) used in the crop rotation systems can release specific compounds in the soil and may lead to changes in the composition of

microbial communities integrated with the crop type (Chavarría et al., 2016; Zhang et al., 2014). However, our results did not indicate differences when winter (R1) or winter/summer (R2) cover crops were employed in the cropping system.

Nonetheless, results demonstrate the importance of cover crops and different species into the cropping system since the monocropping (wheat/soybean (R0)) treatment had the lowest microbial biomass, regardless of soil management. This behavior was similar for all PLFA groups tested. The difference in the input of C and N may have been the driver for these differences since we found a higher C and N content in the R2 (C: 3.05%) treatment in relation to the R0 (C: 2.68%). Campos et al. (2011), working in the same experiment, reported that the soil C input increases with the diversification of the cropping systems, where the intensive cropping system (R2) had the highest C input and monoculture the lowest.

Moreover, the presence of roots of cover crops during the intercrop period can releases exudates in the soil (Jones, 1998; Loveland and Webb, 2003; Zhang et al., 2017). For instance, exudates of low molecular weight compounds such as organic acids, amino acids, sugars and vitamins and compounds of high molecular weight such as polysaccharides (mucilage) and proteins (Kamilova et al., 2006; Haichar et al., 2014; Chavarría et al., 2016) are acting in favor of the growing microbial biomass. Therefore, the continuous and diversified (R2) presence of live roots in the system is an essential step for greater soil health, above all for an increase in the microbial community in agroecosystems. Plant cover during the whole year for greater C and N input and live roots can improve the microbial community and increase the population of bacteria such as Gram+, Gram-, and Actinomycetes as well as fungi such as AMF, and saprophytic fungi.

Furthermore, in subtropical regions, the maintenance of soil moisture and temperature during the intercrop periods are a vital factor for the microbial community structure (Brockett et al., 2012). Straw from the previous crop and the shoot biomass of the crops that are growing under NT, is indispensable for the protection of the soil and the microbial community, conserving soil moisture and reducing soil temperature during the summer (Wang et al., 2011; De Quadros et al., 2012). Studying in the same filed, Pes et al. (2011) reported a lower soil temperature and a higher water-filled porosity in the NT than in the CT, helping to show the effectiveness of NT to maintain temperature and soil moisture.

The long-term NT was the dominant effect for a greater microbial community at 0 to 5 cm. The highest microbial biomass and abundance of total bacteria and fungi in this layer were obtained due to the concentration of C and N (Allison et al., 2007; Amado et al., 2006; Fabrizzi et al., 2003; Mikha and Rice, 2004). In the shallow layer, microbial community was negatively affected by tillage due to several abiotic factors such as changes in chemical, physical, and biological properties (Table 1), modifying their habitat (Mbuthia et al., 2015; Reichert et al., 2016b; van Capelle et al., 2012). Disturbance affected the connectivity of species and disrupt fungal networks (Smith et al., 2016). Moreover, tillage exposes the soil to most frequently wetting and drying cycles, thus increasing soil degradation, organic matter decomposition, and changes in the microbial community (Vezzani and Mielniczuk, 2009; Tivet et al., 2013). Saprophytic fungi, as well as AMF, were the communities more impacted by soil disturbance. CT system induced a reduction of 38.5% of total fungi in relation to NT in the shallow layer while AMF was 38.6% decreased (Table 3). Soil tillage is expected to inhibit fungal growth and proliferation which contribute to macroaggregate formation and preservation. Also, the excretion of mucilage by AMF

such as glomalin contributes to the microaggregates formation (Rashid et al., 2016; Sá et al., 2015; Smith et al., 2000). Thus, it is critical to make use practices such as NT to promote fungi and AMF growth. After six years of the experiment establishment, was possible to verify an increase in soil macroaggregates under NT in relation to the CT (Campos et al., 1995). Furthermore, Campos et al. (1995), reported that the crop rotation increased the soil aggregation when compared with crop succession (R0). In the same experiment, Fabrizzi et al. (2009) documented that NT had 75% more large macroaggregates (>2000 µm) than the tilled treatment, which had more smaller aggregates.

Overall, NT increased AMF and fungi abundance by 38.7% and 38.3%, respectively, in the soil surface, contributing to the maintenance of the fungal hyphal network. The higher fungal and bacterial biomass in the NT was associated with three factors: minimal soil disturbance, higher soil moisture, and lower soil temperature. Lower disturbance in the soil promotes growth and activity of fungi due to the establishment of extensive hyphal networks. Soil cover allows fungi to establish bridges at the soil-stover interface facilitating residue decomposition and soil organic matter formation. Continuous soil disturbance decreases the capacity of soil ecosystem services (Doran and Parkin, 1994; Karlen et al., 1997). Furthermore, agricultural environments that favor fungal growth promote soil carbon storage and preservation through physical protection in macroaggregates (Fabrizzi et al., 2009; Six et al., 2006, 2002).

As expected, the abundance of Gram- bacteria were 53% higher at 0 to 5 cm than to the 10 to 30 cm in NT soil. No-till system provides straw (i.e. plant material) in the soil surface and Gram- bacteria are more adapted to use plant material as C resource. However, Gram+ bacteria abundance was relatively higher with depth and

in the surface in relation to the Gram- bacteria. The feed mechanism can explain this result since they are adapted to use soil organic matter as C resource (Ai et al., 2018; Hsiao et al., 2018; Wan et al., 2014). Furthermore, Gram+ bacteria have a thick wall of peptidoglycan and the ability to produce resistance spores. This condition affords the endurance to environmental stresses such as wetting and drying cycles, supporting its greater abundance at 0 to 5 cm.

Actinomycetes develop extensive mycelia and use C from crop residues. Thus, the most content of actinomycetes stays on the surface, decreasing 48% until 10 to 30 cm. Actinomycetes, as well as fungi, contribute significantly to degradation of recalcitrant carbohydrates like cellulose since they have a wide range of extracellular enzymes (Lu et al., 2017). Moreover, soil fungi are more active than bacteria in response to crop-type conversion (Ai et al., 2018).

Changes in extracellular enzyme activity

The majority of the variation in the βG, NAG, and AcidP activity was explained by soil management, cropping system, and depth (Fig. 3). These three hydrolytic enzymes are responsible for mediating C, N, and P cycling in the soil, respectively (Bowles et al., 2014; Cusack et al., 2011; Zhao et al., 2016). The potential activities of these enzymes are frequently linked to microbial biomass and used as an indicator of microbial C, N and P demand (Sinsabaugh et al., 2009).

βG has been one of the most studied hydrolytic enzymes due to its link to the soil C cycle (Bowles et al., 2014). The βG operates in the decomposition of cellulose (Grandy et al., 2007; Stone et al., 2014). Since cellobiose is a disaccharide of fast decomposition in the soil (Liu et al., 2016), its presence may explain the strong positive correlation among the activity of βG and the C content (Appendix A)

(Matsuoka et al., 2003). Thus, βG is an indicator of the soil system condition due to its rapid response to the changes in the soil use and management (Peixoto et al., 2010).

Under NT, the increase in crop rotation diversity was followed by a rising in the βG activity. The crop residues from cover crops and maize (NTR2) and the proportion of readily decomposable organic components returned to the soil from these crops likely enhanced βG activity. In this case, the βG activity was 48.49% and 29.9% higher in relation to the crop succession (NTR0) and winter rotation (NTR1), respectively. Soil disturbance by CT decrease homogeneously βG activity in depth and overlaps the effect of crop diversity within the cropping system. The more oxidative biochemical microhabitats of the CT soils can support this finding (Zhang et al., 2014).

Tillage had a profound effect on soil enzymes. βG, NAG, and AcidP were significantly higher in the NT than CT treatments, excluding CTR2 for AcidP. Similar results have been reported by several other studies (Shi et al., 2013; Zhang et al., 2014; Zuber and Villamil, 2016). Synthesis of extracellular enzymes is induced by the presence of their substrates (Nannipieri et al., 2012). Thus, the higher activity of the hydrolytic enzymes in NT indicate greater availability of cellulose and nutrients such as C, N, and Ca^{2+} (Blanco-Canqui et al., 2009; de Oliveira Ferreira et al., 2018; Six and Paustian, 2014). The presence of substrates from multiple crops in the diversified rotation promoted the greater hydrolytic activity.

AcidP activity facilitates organic P mineralization into phosphate by hydrolyzing phosphoric ester bonds under acid conditions (German et al., 2011; Grandy et al., 2007). In most cases, P deficiency in the soil can stimulate the AcidP activity. However, this theory could not be supported by our results. Furthermore, we

did not find a correlation between AcidP activity and P content (Appendix B). Even though our results are not related to the content of P, diversified cropping systems with cash crops and cover crops such as R2 treatment may increase P demand, inducing the AcidP to a higher activity. This finding is in agreement with Bell et al. (2008) since the highest AcidP activities were reported in the NTR2, NTR1, and CTR2 treatments, respectively. Moreover, the C content can explain the higher AcidP activity in the NTR1 and NTR2 treatments. Soil with higher soil organic carbon content is considered to encompass more organic matter, which can be a considerable proxy of organic P corroborating with our results (Table 1; Appendix B) (Margalef et al., 2017).

Conversely, NAG activity was not correlated with either C or N content. The NAG activity was only weakly negatively correlated with clay (Appendix B). NAG had the higher activities under NT in the R1 and R2 cropping systems. Nonetheless, over crops successions – soybeans/wheat monoculture (R0), there was a decrease of NAG activity at 5 to 10 cm, presumably due to the lack of crop diversity and lower microbial biomass. Stone et al., (2014), reported that in deep soils, such as Oxisols, bacterially-derived NAG enzymes play an indispensable role in recycling organic N from microbial biomass. NAG is linked to the hydrolysis of terminal 1,4 linked N-acetyl-beta-D-glucosaminide residues in chitooligosaccharides (that is, chitin degradation) (German et al., 2011; Grandy et al., 2007), and is involved in the degradation of peptidoglycan, a component of bacterial cell walls (Stone et al., 2014). Thus, due to the importance of these enzymes in the catalysis of substrates in organic compounds, the hydrolytic enzyme activity can be an indicator of soil health.

Relative influence of soil physicochemical attributes on microbial community and enzyme activity

Based on the dbRDA, the variation in the microbial community and enzyme activity could be explained by soil management and then by cropping system. Clustering clearly separated microbial and physicochemical properties correlated with NT or CT in the upland and lowland, respectively. Results from the Monte Carlos permutation indicated that pH and Ca^{2+} were crucial abiotic factors regulating microbial communities. Soils from tropical and subtropical regions are acids and rich in Al^{+3}. In subtropical conditions, the soil correction for acidity (liming) must be considered an essential tool to improve the chemical quality and increase the microbial biomass, as well as crop yield, of these soils (Mühlbachová and Tlustoš, 2006; Xue et al., 2010). The microbial community and enzyme activity were positively correlated with P, N, C, Mg^{+2}, Ca^{2+}, and negatively correlated with Al^{+3}. The absence of soil disturbance and increase diversity of crop rotation created better conditions for the microbial community at 0 to 5 cm. Moreover, NT had higher pH and Ca^{2+} content than CT at 0 to 5 cm (Table 1).

In the subsurface, at 10 to 30 cm, the variability of the microbial community and enzyme activity was positively correlated with pH, Ca^{2+}, Mg^{2+}, C, N, CEC and EC and negatively correlated with Al^{3+} and clay. Microbial biomass, total fungi and, total bacteria were higher in the CT than NT at the 10-30 cm depth. Thus, these results strengthen the importance of soil acidity correction, since the NT can create a gradient of fertility impairing root development and decreasing exudates release on the subsurface (Dalla Nora and Amado, 2013), causing a decline in the microbial biomass. Furthermore, the residue incorporation under CT increased all microbial communities accessed by PLFA in deeper layers. Outstandingly, excluding the

amount, the deployment of the microbial community under NT at 0 to 5 cm resembled the microbial community under CT at 10-30 cm.

Therefore, our study is in agreement and reinforces that Oxisols handled under NT has created a strong nutrient stratification over time. Also, our results portrayed that all the microbial communities accessed and the activity of the enzymes were moderated by abiotic factors, such as cropping system variety and soil management. These two factors modified nutrients (Ca^{2+}, Mg^{2+}, and P), C, and N content, thus regulating the soil microbiome.

Nonetheless, despite incorporating crop residues into the subsurface, frequently tillage practices such as chisel plow, disk plow, and disk harrow, make the soil system dissipate energy and unbalance, improving entropy process (Addiscott, 1995; Reichert et al., 2016b). Otherwise, when adopting conservation practices such as long-term NT and cropping systems provided with diversified plan species (matter input), the soil environment tends to an organization. This organizational status reflects in a structured, equilibrated and healthy soil, provided with a robust microbial community and enzyme activity, being able to cycle nutrients, increase productive capacity and to accomplish its functions.

Conclusions

We investigated the effect of the soil management and cropping systems over soil microbial communities, and extracellular enzymes activity knows to regulate carbon and nutrient cycling in agricultural lands. Long-term NT was the key factor controlling the soil microbial communities regardless of crop rotation system. As predicted, tillage had a profound effect on biomass and composition of soil microbial communities as well as enzymes activity. Long-term NT increased microbial biomass

as evaluated by total PLFA biomarkers in the topsoil (0 to 5 cm). In contrast, soil microbial communities were augmented in deeper soil layers (10 to 30 cm) of the CT soil. Nonetheless, the use of crop rotation with either summer or winter diversified crops increased all tested microbial communities as evaluated by specific bacteria (total, Gram+, Gram-), actinomycetes, and fungi (total, AMF and, fungi) biomarkers in comparison with wheat/soybean cropping system. Soil β-glucosidase, acid phosphatase, and N-acetyl-glucosaminidase enzymes activity were more sensitive to the interaction of soil management and cropping systems than microbial communities biomarkers. The association of NT and increased crop rotation augmented the activity of all three enzymes in the soil surface. Nevertheless, differences among cropping systems decreased with soil depth and were discrete under CT.

Distance-based redundancy analysis revealed that β-glucosidase and N-acetyl-glucosaminidase were closely related with the concentration of their specific substrates (C and N) in the 0 to 5 cm m soil layer. In contrast, no clear correlation between acid phosphatase and soil P test levels were identified. The higher abundance of microbial groups in the 0 to 5 cm and 10 to 30 cm layers of respective NT and CT soils were correlated with increased C and nutrient levels (N, Ca^{2+}, Mg^{2+}) and decreased Al^{3+} concentrations, as a result of residue and nutrient retention on the surface of NT soils and incorporation within the plow layer in CT soils. Our results suggest that amelioration of subsoil acidity and fertility is the pathway to enhancing microbial biomass in deeper soil layers of NT soils. Our analysis also showed that increase on crop rotation diversity favored the abundance of both microbial communities and extracellular enzymes activity in the surface of NT soils, thus supporting that reduced soil disruption and agricultural intensification may increase soil health and nutrient cycling in agroecosystems.

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APÊNDICE

APÊNDICE A – Analysis of variance of the effect of soil management, cropping system, depth, and their respective interactions on the soil microbial properties.

Type III Tests of Fixed Effects

Microbial biomass			
Source of Variation	denDF	F-value	p-value
Tillage (T)	12	0.0804	0.7817
Crop Rotation (CR)	12	6.4227	0.0127
Depth (D)	24	43.4172	<.0001
T*CR	12	0.4433	0.652
T*D	24	26.5995	<.0001
CR*D	24	1.0831	0.387
T*CR*D	24	1.2003	0.3363

Total bacteria			
Source of Variation	denDF	F-value	p-value
Tillage (T)	12	0.1056	0.7508
Crop Rotation (CR)	12	8.5966	0.0048
Depth (D)	24	37.3262	<.0001
T*CR	12	0.6634	0.533
T*D	24	18.3297	<.0001
CR*D	24	0.8734	0.4943
T*CR*D	24	0.8163	0.5273

Total fungi			
Source of Variation	denDF	F-value	p-value
Tillage (T)	12	0.1151	0.7403
Crop Rotation (CR)	12	6.074	0.0151
Depth (D)	24	86.436	<.0001
T*CR	12	0.3021	0.7447
T*D	24	46.9465	<.0001
CR*D	24	1.4099	0.2608
T*CR*D	24	1.9167	0.1403

Gram-positive bacteria			
Source of Variation	denDF	F-value	p-value
Tillage (T)	12	0.87	0.3693
Crop Rotation (CR)	12	7.5321	0.0036
Depth (D)	24	41.6836	<.0001
T*CR	12	0.6593	0.535
T*D	24	21.9697	<.0001
CR*D	24	0.7435	0.5718
T*CR*D	24	0.9556	0.4496

Gram-negative bacteria			
Source of Variation	denDF	F-value	p-value
Tillage (T)	12	0.1531	0.7024
Crop Rotation (CR)	12	8.1551	0.0058
Depth (D)	24	32.9105	<.0001
T*CR	12	0.5403	0.5961
T*D	24	12.8557	<.0001
CR*D	24	1.2474	0.3178
T*CR*D	24	0.656	0.6284

Actinomycetes			
Source of Variation	denDF	F-value	p-value
Tillage (T)	12	0.4418	0.5188
Crop Rotation (CR)	12	6.8154	0.0105
Depth (D)	24	22.2066	<.0001
T*CR	12	0.4659	0.6384
T*D	24	13.7292	<.0001
CR*D	24	1.0891	0.3842
T*CR*D	24	0.6605	0.6254

Fungi			
Source of Variation	denDF	F-value	p-value
Tillage (T)	12	0.0457	0.8344
Crop Rotation (CR)	12	4.6691	0.0316
Depth (D)	24	45.3968	<.0001
T*CR	12	0.6475	0.5407
T*D	24	18.3306	<.0001
CR*D	24	1.8205	0.1578
T*CR*D	24	0.744	0.5715

Arbuscular mycorrhizal fungi			
Source of Variation	denDF	F-value	p-value
Tillage (T)	12	0.3426	0.5692
Crop Rotation (CR)	12	6.4591	0.0125
Depth (D)	24	111.7125	<.0001
T*CR	12	0.2142	0.8102
T*D	24	67.217	<.0001
CR*D	24	1.2188	0.3289
T*CR*D	24	2.8992	0.0533

β-glucosidase			
Source of Variation	denDF	F-value	p-value
Tillage (T)	12	182.011	<.0001
Crop Rotation (CR)	12	10.5806	0.0022
Depth (D)	24	384.059	<.0001
T*CR	12	13.1064	0.001
T*D	24	277.239	<.0001
CR*D	24	9.0649	0.0001
T*CR*D	24	6.5849	0.001

Acid phosphatase			
Source of Variation	denDF	F-value	p-value
Tillage (T)	12	0.023	0.8809
Crop Rotation (CR)	12	18.278	0.0002
Depth (D)	24	24.561	<.0001
T*CR	12	4.793	0.0295
T*D	24	23.716	<.0001
CR*D	24	7.355	0.0005
T*CR*D	24	4.873	0.0051

N-acetyl-glucosaminidase			
Source of Variation	denDF	F-value	p-value

Tillage (T)	12	101.235	<.0001
Crop Rotation (CR)	12	15.5311	0.0005
Depth (D)	24	0.877	0.429
T*CR	12	12.5656	0.0011
T*D	24	10.4421	0.0005
CR*D	24	2.6655	0.057
T*CR*D	24	0.4858	0.746

APÊNDICE B – Pearson correlation coefficients for studied variables. NS is not statistically significant at α = 0.05. Values in bold are significant at α = <0.001.

	N	C	pH	Ca^{2+}	Mg^{2+}	Al^{3+}	K^{+}	P	CEC	EC	Clay	MB	Gram+	Gram-	Actino	AMF	Fungi	βG	AcidP	NAG
N	1.000																			
C	**0.968**	1.000																		
pH	**0.547**	**0.564**	1.000																	
Ca^{2+}	**0.571**	**0.570**	**0.791**	1.000																
Mg^{2+}	**0.583**	**0.583**	**0.758**	**0.901**	1.000															
Al^{3+}	-0.384	-0.391	**-0.708**	**-0.671**	**-0.637**	1.000														
K^{+}	0.310	0.313	0.370	**0.455**	**0.450**	-0.310	1.000													
P	**0.450**	**0.455**	**0.577**	**0.502**	0.426	NS	0.397	1.000												
CEC	0.335	0.386	**0.521**	**0.549**	**0.486**	-0.291	**0.473**	**0.497**	1.000											
EC	**0.815**	**0.873**	**0.470**	**0.559**	**0.583**	-0.298	0.367	**0.488**	0.380	1.000										
Clay	**-0.674**	**-0.661**	**-0.608**	**-0.617**	**-0.537**	0.417	NS	**-0.586**	-0.370	**-0.645**	1.000									
MB	**0.814**	**0.799**	0.408	**0.568**	**0.557**	-0.398	**0.501**	**0.437**	0.352	**0.822**	**-0.693**	1.000								
Gram+	**0.781**	**0.760**	0.414	**0.596**	**0.570**	**-0.441**	**0.518**	0.421	0.376	**0.764**	**-0.664**	**0.983**	1.000							
Gram-	**0.749**	**0.738**	NS	**0.462**	**0.470**	-0.278	0.418	0.340	0.324	**0.773**	**-0.577**	**0.946**	**0.942**	1.000						
Actino	**0.722**	**0.708**	NS	0.383	0.400	NS	0.365	0.319	NS	**0.752**	**-0.567**	**0.939**	**0.916**	**0.979**	1.000					
AMF	**0.828**	**0.814**	**0.441**	**0.556**	**0.559**	-0.417	0.432	**0.513**	0.364	**0.813**	**-0.688**	**0.957**	**0.946**	**0.926**	**0.918**	1.000				
Fungi	**0.816**	**0.807**	0.378	**0.558**	**0.565**	-0.382	**0.534**	0.413	0.336	**0.817**	**-0.639**	**0.980**	**0.964**	**0.950**	**0.924**	**0.933**	1.000			
βG	**0.872**	**0.915**	**0.592**	**0.598**	**0.602**	-0.380	NS	**0.474**	0.396	**0.907**	**-0.723**	**0.776**	**0.739**	**0.713**	**0.675**	**0.789**	**0.763**	1.000		
AcidP	**0.625**	**0.612**	NS	0.368	0.414	-0.276	0.300	NS	NS	**0.659**	**-0.485**	**0.708**	**0.684**	**0.680**	**0.659**	**0.672**	**0.710**	**0.654**	1.000	
NAG	NS	NS	NS	NS	NS	NS	-0.368	NS	NS	0.352	-0.285	NS	NS	NS	NS	NS	NS	**0.436**	NS	1.000

APÊNDICE C – Mean and standard error (S.E.) of the microbial properties studied.

Microbial biomass				
Tillage	Crop Rotation	Depth (m)	Mean	S.E.
CT	R0	0.00_0.05	23.49	1.72
CT	R0	0.05_0.10	20.93	0.29
CT	R0	0.10_0.30	17.89	1.55
CT	R1	0.00_0.05	24.53	4.44
CT	R1	0.05_0.10	26.88	2.25
CT	R1	0.10_0.30	27.52	4.76
CT	R2	0.00_0.05	28.22	1.91
CT	R2	0.05_0.10	26.26	1.16
CT	R2	0.10_0.30	18.78	4.71
NT	R0	0.00_0.05	31.38	0.82
NT	R0	0.05_0.10	15.52	3.20
NT	R0	0.10_0.30	10.60	0.40
NT	R1	0.00_0.05	44.99	2.87
NT	R1	0.05_0.10	21.75	2.55
NT	R1	0.10_0.30	14.85	3.78
NT	R2	0.00_0.05	44.19	6.36
NT	R2	0.05_0.10	23.00	3.75
NT	R2	0.10_0.30	12.43	2.29

Total bacteria				
Tillage	Crop Rotation	Depth (m)	Mean	S.E.
CT	R0	0.00_0.05	9.93	0.61
CT	R0	0.05_0.10	9.03	0.05
CT	R0	0.10_0.30	7.79	0.45
CT	R1	0.00_0.05	10.56	1.71
CT	R1	0.05_0.10	11.04	0.79
CT	R1	0.10_0.30	10.45	1.25
CT	R2	0.00_0.05	11.85	0.87
CT	R2	0.05_0.10	11.34	0.47
CT	R2	0.10_0.30	8.42	1.84
NT	R0	0.00_0.05	11.76	0.41
NT	R0	0.05_0.10	6.89	1.17
NT	R0	0.10_0.30	4.97	0.20
NT	R1	0.00_0.05	16.98	1.24
NT	R1	0.05_0.10	9.26	1.19
NT	R1	0.10_0.30	6.75	1.21
NT	R2	0.00_0.05	16.51	2.04
NT	R2	0.05_0.10	9.95	1.34
NT	R2	0.10_0.30	5.77	0.95

Total fungi				
Tillage	Crop Rotation	Depth (m)	Mean	S.E.
CT	R0	0.00_0.05	5.20	0.39
CT	R0	0.05_0.10	4.50	0.07
CT	R0	0.10_0.30	3.77	0.44
CT	R1	0.00_0.05	5.29	0.46
CT	R1	0.05_0.10	6.00	0.67
CT	R1	0.10_0.30	5.51	0.90
CT	R2	0.00_0.05	6.35	0.32
CT	R2	0.05_0.10	5.87	0.46
CT	R2	0.10_0.30	3.93	0.80
NT	R0	0.00_0.05	7.58	0.23
NT	R0	0.05_0.10	3.29	0.79
NT	R0	0.10_0.30	1.91	0.06
NT	R1	0.00_0.05	10.24	0.60
NT	R1	0.05_0.10	4.57	0.49
NT	R1	0.10_0.30	2.48	0.68
NT	R2	0.00_0.05	9.64	1.08
NT	R2	0.05_0.10	5.52	0.85
NT	R2	0.10_0.30	2.26	0.49

Gram-positive bacteria				
Tillage	Crop Rotation	Depth (m)	Mean	S.E.
CT	R0	0.00_0.05	5.24	0.36
CT	R0	0.05_0.10	4.74	3.70E-03
CT	R0	0.10_0.30	4.04	0.16
CT	R1	0.00_0.05	5.73	1.25
CT	R1	0.05_0.10	5.80	0.53
CT	R1	0.10_0.30	5.60	0.72
CT	R2	0.00_0.05	6.16	0.53
CT	R2	0.05_0.10	5.83	0.23
CT	R2	0.10_0.30	4.21	1.11
NT	R0	0.00_0.05	6.36	0.20
NT	R0	0.05_0.10	3.18	0.59
NT	R0	0.10_0.30	2.09	0.12
NT	R1	0.00_0.05	9.63	0.68
NT	R1	0.05_0.10	4.29	0.57
NT	R1	0.10_0.30	3.01	0.68
NT	R2	0.00_0.05	8.98	1.29
NT	R2	0.05_0.10	4.56	0.54
NT	R2	0.10_0.30	2.63	0.44

Gram-negative bacteria				
Tillage	Crop Rotation	Depth (m)	Mean	S.E.
CT	R0	0.00_0.05	3.69	0.20
CT	R0	0.05_0.10	3.30	0.03
CT	R0	0.10_0.30	2.90	0.24
CT	R1	0.00_0.05	3.79	0.39
CT	R1	0.05_0.10	4.07	0.21
CT	R1	0.10_0.30	3.72	0.49
CT	R2	0.00_0.05	4.50	0.25
CT	R2	0.05_0.10	4.30	0.20
CT	R2	0.10_0.30	3.27	0.58
NT	R0	0.00_0.05	4.21	0.17
NT	R0	0.05_0.10	2.85	0.46
NT	R0	0.10_0.30	2.20	0.07
NT	R1	0.00_0.05	5.74	0.44
NT	R1	0.05_0.10	3.83	0.48
NT	R1	0.10_0.30	2.86	0.41
NT	R2	0.00_0.05	5.96	0.63

Actinomycetes				
Tillage	Crop Rotation	Depth (m)	Mean	S.E.
CT	R0	0.00_0.05	0.99	0.06
CT	R0	0.05_0.10	0.98	0.01
CT	R0	0.10_0.30	0.86	0.08
CT	R1	0.00_0.05	1.03	0.07
CT	R1	0.05_0.10	1.17	0.06
CT	R1	0.10_0.30	1.13	0.11
CT	R2	0.00_0.05	1.19	0.10
CT	R2	0.05_0.10	1.21	0.04
CT	R2	0.10_0.30	0.94	0.15
NT	R0	0.00_0.05	1.19	0.07
NT	R0	0.05_0.10	0.86	0.14
NT	R0	0.10_0.30	0.68	0.01
NT	R1	0.00_0.05	1.61	0.15
NT	R1	0.05_0.10	1.14	0.17
NT	R1	0.10_0.30	0.87	0.12
NT	R2	0.00_0.05	1.57	0.13

NT	R2	0.05_0.10	4.19	0.65
NT	R2	0.10_0.30	2.43	0.40

Arbuscular mycorrhizal fungi

Tillage	Crop Rotation	Depth (m)	Mean	S.E.
CT	R0	0.00_0.05	3.69	0.28
CT	R0	0.05_0.10	3.33	0.03
CT	R0	0.10_0.30	2.79	0.27
CT	R1	0.00_0.05	3.77	0.21
CT	R1	0.05_0.10	4.24	0.40
CT	R1	0.10_0.30	3.90	0.56
CT	R2	0.00_0.05	4.34	0.20
CT	R2	0.05_0.10	4.13	0.36
CT	R2	0.10_0.30	3.03	0.46
NT	R0	0.00_0.05	5.62	0.18
NT	R0	0.05_0.10	2.46	0.56
NT	R0	0.10_0.30	1.53	0.05
NT	R1	0.00_0.05	7.30	0.37
NT	R1	0.05_0.10	3.44	0.32
NT	R1	0.10_0.30	1.85	0.38
NT	R2	0.00_0.05	6.31	0.42
NT	R2	0.05_0.10	4.02	0.49
NT	R2	0.10_0.30	1.79	0.36

β-glucosidase

Tillage	Crop Rotation	Depth (m)	Mean	S.E.
CT	R0	0.00_0.05	74.69	6.75
CT	R0	0.05_0.10	65.45	2.48
CT	R0	0.10_0.30	58.03	5.35
CT	R1	0.00_0.05	73.91	6.75
CT	R1	0.05_0.10	68.84	8.28
CT	R1	0.10_0.30	59.77	9.37
CT	R2	0.00_0.05	76.10	2.76
CT	R2	0.05_0.10	65.41	5.94
CT	R2	0.10_0.30	48.74	13.25
NT	R0	0.00_0.05	202.7	5.46
NT	R0	0.05_0.10	70.74	7.30
NT	R0	0.10_0.30	27.99	1.03
NT	R1	0.00_0.05	231.7	13.33
NT	R1	0.05_0.10	59.12	11.43
NT	R1	0.10_0.30	80.69	10.03
NT	R2	0.00_0.05	301.0	9.63
NT	R2	0.05_0.10	95.69	4.58
NT	R2	0.10_0.30	63.61	4.63

N-acetyl-glucosaminidase

Tillage	Crop Rotation	Depth (m)	Mean	S.E.
CT	R0	0.00_0.05	62.88	10.38
CT	R0	0.05_0.10	73.25	4.09
CT	R0	0.10_0.30	75.03	3.25
CT	R1	0.00_0.05	58.60	3.25
CT	R1	0.05_0.10	61.38	9.73
CT	R1	0.10_0.30	82.38	11.24
CT	R2	0.00_0.05	62.15	3.96
CT	R2	0.05_0.10	67.04	3.99
CT	R2	0.10_0.30	96.37	3.29
NT	R0	0.00_0.05	95.84	8.28
NT	R0	0.05_0.10	94.85	14.56
NT	R0	0.10_0.30	60.40	10.40
NT	R1	0.00_0.05	120.0	6.50
NT	R1	0.05_0.10	126.8	6.97
NT	R1	0.10_0.30	124.1	13.74
NT	R2	0.00_0.05	139.7	4.77
NT	R2	0.05_0.10	1.20	0.18
NT	R2	0.10_0.30	0.71	0.12

Fungi

Tillage	Crop Rotation	Depth (m)	Mean	S.E.
CT	R0	0.00_0.05	1.51	0.12
CT	R0	0.05_0.10	1.16	0.04
CT	R0	0.10_0.30	0.98	0.17
CT	R1	0.00_0.05	1.53	0.30
CT	R1	0.05_0.10	1.76	0.29
CT	R1	0.10_0.30	1.61	0.37
CT	R2	0.00_0.05	2.02	0.17
CT	R2	0.05_0.10	1.74	0.13
CT	R2	0.10_0.30	0.90	0.34
NT	R0	0.00_0.05	1.96	0.07
NT	R0	0.05_0.10	0.83	0.23
NT	R0	0.10_0.30	0.38	0.02
NT	R1	0.00_0.05	2.94	0.24
NT	R1	0.05_0.10	1.13	0.17
NT	R1	0.10_0.30	0.63	0.29
NT	R2	0.00_0.05	3.32	0.68
NT	R2	0.05_0.10	1.50	0.36
NT	R2	0.10_0.30	0.47	0.13

Acid phosphatase

Tillage	Crop Rotation	Depth (m)	Mean	S.E.
CT	R0	0.00_0.05	323.26	6.04
CT	R0	0.05_0.10	331.70	3.27
CT	R0	0.10_0.30	330.90	21.43
CT	R1	0.00_0.05	323.70	8.88
CT	R1	0.05_0.10	339.21	12.19
CT	R1	0.10_0.30	356.07	11.67
CT	R2	0.00_0.05	374.54	4.80
CT	R2	0.05_0.10	337.28	9.96
CT	R2	0.10_0.30	345.75	6.95
NT	R0	0.00_0.05	328.90	5.29
NT	R0	0.05_0.10	270.96	11.51
NT	R0	0.10_0.30	305.90	13.66
NT	R1	0.00_0.05	409.43	3.65
NT	R1	0.05_0.10	307.18	12.13
NT	R1	0.10_0.30	344.88	7.44
NT	R2	0.00_0.05	428.18	12.62
NT	R2	0.05_0.10	360.18	22.86
NT	R2	0.10_0.30	298.64	6.65

NT	R2	0.05_0.10	141.5	8.43
NT	R2	0.10_0.30	133.5	5.05