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LUCIANE CRISTINA LAZZARIN

MICROBIOLOGIA DO SOLO E ESTOQUES DE CARBONO E NITROGÊNIO EM SISTEMAS INTEGRADOS DE PRODUÇÃO AGROPECUÁRIA COM FERTILIZANTES ORGÂNICOS OU MINERAIS

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Orientador: Iraê Amaral Guerrini
Coorientador: Juliano Corulli Corrêa

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TÍTULO DA TESE: MICROBIOLOGIA DO SOLO E ESTOQUES DE CARBONO E NITROGÊNIO EM SISTEMAS INTEGRADOS DE PRODUÇÃO AGROPECUÁRIA COM FERTILIZANTES ORGÂNICOS E MINERAIS

AUTORA: LUCIANE CRISTINA LAZZARIN
ORIENTADOR: IRAÊ AMARAL GUERRINI
COORIENTADOR: JULIANO CORULLI CORRÊA

Aprovada como parte das exigências para obtenção do Título de Doutora em CIÊNCIA FLORESTAL, pela Comissão Examinadora:

Prof. Dr. IRAÊ AMARAL GUERRINI (Participaçao Virtual)
Ciência Florestal, Solos e Ambiente / Faculdade de Ciências Agronômicas de Botucatu - UNESP

P/ Prof. Dr. JOSÉ RICARDO MACEDO PEZZOPANE (Participaçao Virtual)
Pesquisa e Desenvolvimento / Embrapa Pecuária Sudeste

P/ Prof.ª Dr.ª GISLAINE FONGARO (Participaçao Virtual) Microbiologia / Universidade Federal de Santa Catarina

P/ Prof. Dr. JULIANO CARLOS CALONEGO (Participaçao Virtual)
Produção Vegetal / Faculdade de Ciências Agronômicas de Botucatu - UNESP

P/ Prof.ª Dr.ª ALINE VIANCELLI (Participaçao Virtual)
Microbiologia / Universidade do Contestado

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Às minhas filhas, Natalia e Isabela,
razões da minha existência,

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RESUMO

Os sistemas integrados de produção são alternativas promissoras para a agricultura sustentável, permitindo uma interação positiva entre animais e plantas, o que resulta em benefícios ambientais. Os objetivos deste trabalho foram investigar a contribuição dos fertilizantes orgânicos e minerais no sequestro de carbono e nas propriedades microbiológicas do solo em dois sistemas integrados de produção agropecuária. Os tratamentos incluíram dois sistemas de produção conservacionista: Integração Lavoura-Pecuária (iLP) e Integração Lavoura-Pecuária-Floresta (iLPF), além da interação com três diferentes fertilizantes (dejeto suíno, cama de aves e inorgânico) e o controle sem fertilização. Para a análise dos teores de C e N aportados pelas culturas, amostras de solo foram coletadas em trincheiras e analisadas através do analisador elementar (CHN) e as análises microbiológicas seguiram a metodologia PLFA. Maior eficiência no aporte de C em 2018 foi encontrada no sistema iLP (2590 kg ha^{-1}) sobre o iLPF (2030 kg ha^{-1}) no tratamento controle, e nenhuma outra diferença foi encontrada comparando os sistemas de produção. Em relação aos fertilizantes, as maiores contribuições de C e N em 2018 foram observadas no solo fertilizado com cama de aves e dejeto de suínos, com eficiência de 56% para C no sistema iLP e 59% no iLPF, e 60% para N no iLP e 62% no iLPF. Porém, em 2019 o fertilizante inorgânico apresentou diferença no iLP e cama de aves no iLPF (ambos com eficiência da ordem de 27%), tanto para C quanto para N. Considerando o perfil total do solo na comparação entre os sistemas de produção, o sistema iLPF apresentou eficiência de 20,7% sobre o iLP em relação ao estoque de C no tratamento controle, e o estoque de N foi mais eficiente sobre o iLP na cama de frango e fertilizantes minerais. Quando não há fertilização nos sistemas de produção, o iLPF é superior ao iLP para aporte de C, no entanto, quando há fertilização com cama de aves e inorgânico, o sistema iLP é superior ao iLPF para aporte de N. O fertilizante inorgânico ou orgânico aumentou a entrada de C apenas no sistema iLP, demonstrando que há outra dinâmica de acúmulo de C quando o eucalipto está presente no sistema iLPF. A concentração microbiana foi maior na primeira camada do solo, sendo Gram-negativas a fração majoritária. Os resultados destacam os benefícios dos sistemas agroflorestais para a agricultura sustentável.

Palavras-chave: Carbono no solo; Integração lavoura-Pecuária; Integração lavoura-Pecuária-Floresta; biomassa microbiana do solo.

ABSTRACT

Integrated production systems are promising alternatives for sustainable agriculture, allowing a positive interaction between animals and plants, which results in environmental benefits and economic viability. The objectives of this study were to investigate the contribution of organic and inorganic fertilizers to carbon sequestration, and the microbiological properties of the soil in two integrated agricultural production systems. The treatments included two conservation production systems: Crop-Livestock Integration (ICL) and Crop-Livestock-Forest Integration (ICLF) and interaction with three different fertilizers (liquid swine manure, poultry litter and inorganic) and control without fertilization. To verify the content of C and N input by the cultures, soil samples were collected in trenches and analyzed through the elementary analyzer, while the microbiology assay was performed by PLFA. Eucalyptus dendrometric variables were collected to estimate the C content. Greater efficiency in the C input in 2018 was found in the iLP system (2590 kg ha^{-1}) over the ICLF (2030 kg ha^{-1}) in the control treatment, and no other difference was found when comparing the production systems. In relation to fertilizers, the largest contributions of C and N in 2018 were observed in poultry litter and liquid swine manure, with efficiency of 56% for C in the ICL system and 59% in ICLF, and 60% for N in ICL and 62% in ICLF. However, in 2019 the inorganic fertilizer showed difference in ICL and poultry litter in ICLF (both with 27%efficiency) for both C and N. Considering the total soil profile among production systems, ICLF system showed 20.7% efficiency over ICL in relation to the C stock in the control treatment, and the N stock was more efficient over the ICL in poultry litter and inorganic fertilizers. When no fertilization was applied in the production systems, ICLF is superior to ICL for C input, however when fertilization is done with poultry litter and inorganic, the ICL system is superior to ICLF for N input. Inorganic or organic fertilizer increased C input only in the ICLF system, demonstrating that there is another C dynamic when eucalyptus is present in the ICLF system. The concentration of the microbial community was higher in the first soil layer, with Gram-negative micro-organisms as the major fraction. The results highlight the benefits of agroforestry systems for sustainable agriculture.

Keywords: Soil carbon; Crop-Livestock Integration; Crop-Livestock-Forest Integration; soil microbial biomass.

SUMÁRIO

INTRODUÇÃO GERAL

Com a crescente demanda por alimentos, surgem sistemas com maior sustentabilidade de produção, dentre eles os que apresentam integração entre culturas (GOUVELLO, 2010). Os sistemas integrados têm a finalidade de agregar ao uso da terra vários meios de produção, proporcionando diversificação de renda ao produtor (HIRAKURI et al., 2012), melhoria dos atributos químicos e físicos do solo, aumento da ciclagem dos nutrientes e diminuição dos custos de produção (ALVARENGA; GONTIJO NETO, 2010).

Entre os sistemas de integração, estão a integração lavoura- pecuária (iLP) e a integração lavoura-pecuária-floresta (iLPF). Ambos buscam a produção sustentável integrando atividades agrícolas, pecuárias e eventualmente florestais na mesma área. A integração destas atividades ocorre independentemente do tipo de cultivo (consorciado, rotação ou sucessão) e tem várias possibilidades de combinação (BALBINO et al., 2012). Nesse caso o iLPF é o sistema que apresenta maior interação entre essas atividades, com a finalidade de buscar efeitos sinérgicos entre os componentes, contemplando a sustentabilidade ambiental, social e econômica (BALBINO et al., 2011).

O sistema iLPF pode trazer inúmeros benefícios, como financeiros, sociais e ecológicos. A lavoura, um dos componentes do iLPF, gera renda em curto prazo ao produtor e o componente arbóreo também traz vantagens: melhoria na estrutura do solo, aumento na ciclagem de nutrientes, maior conforto térmico aos animais e produtividade elevada do sistema (CAJAS-GIRON; SINCLAIR, 2001). Além disso, o sistema iLPF pode mitigar as emissões dos gases do efeito estufa e aumentar o sequestro de carbono, sobretudo pela introdução das árvores no sistema (OLIVEIRA; VECCHIA, 2013).

A adoção do sistema iLPF coopera com o que foi proposto na COP 21 (21º Conferência das Nações Unidas sobre as Mudanças Climáticas ocorrida em Paris, em 2015), chamado de Acordo de Paris, em que uma das iniciativas é compensar 30% das emissões de gases do efeito estufa (GEE), para segurança alimentar e o desenvolvimento sustentável (LAL et al., 2015; MINASNY et al., 2017). No Brasil, foi criada em 2009 a Política Nacional de Mudanças Climáticas (PNMC), através da Lei nº 12.187, e uma das metas desta política seria a redução das emissões dos gases do efeito estufa entre 36,1% e 38,9% até 2020 (BRASIL, 2009).

O uso de fertilizantes orgânicos em sistemas de produção com integração pode resultar em ganhos econômicos e ambientais, devido a potencialização da diversidade, novas rotas de ciclagem de nutrientes e processos ecossistêmicos (ANGHINONI et al., 2011). Diferentes estudos têm demonstrado que a fertilização com esterco, sozinho ou combinado, aumenta o teor de Carbono Orgânico Total (COT) do solo quando comparado com a fertilização inorgânico (HENTZ et al., 2015; MAFRA et al., 2015). Além disso, a fertilização orgânica, pode trazer benefícios econômicos ao produtor rural, principalmente em razão da redução de custos com aquisição de adubos minerais (MAFRA et al., 2015).

O N está presente na matéria orgânica, logo, o manejo do solo influencia diretamente nos estoques de N total (NT) (SOUZA et al., 2009). Em um experimento de 33 anos no norte da China, com o objetivo de testar diferentes tipos de adubação nas culturas do trigo e milho, foi observado que a aplicação de dejetos orgânicos aliado a adubação com N inorgânico, proporciona aumento nos teores de C e N do sistema, contribuindo para maior produção de grãos (YANG et al., 2015). Em estudo com o objetivo de avaliar os estoques de N no solo em sistema iLPF, durante 13 anos, foi constatado menores perdas de N quando comparado ao sistema de monocultivos convencionais (SACRAMENTO et al., 2013).

A adubação orgânica proporciona aumento dos teores de N no solo, de forma que a determinação dos estoques de N é fundamental para avaliar a sua qualidade. A liberação do N no solo ocorre através dos resíduos vegetais, que através do processo de decomposição pelos microrganismos, parte é imobilizada, parte é disponibilizada para as plantas, uma parte retorna para a atmosfera em forma de gás e o restante é perdido por lixiviação ou liberado para produção de substâncias húmicas (STEVENSON; COLE 1985).

Para que o N e outros nutrientes sejam liberados à solução do solo, se faz necessária a atividade de microrganismos que são responsáveis pela decomposição da matéria orgânica (ÁLVARO-FUENTES et al., 2012) e organização dos nutrientes, que são prontamente disponibilizados para as plantas. Assim, as plantas são dependentes destes microrganismos para que possam absorver os nutrientes que são convertidos para a forma inorgânica (CHEN et al., 2003).

Considerando que tanto os sistemas integrados de produção, a fertilização orgânica e a atividade microbiana têm importantes papéis no estoque de carbono, é imprescindível avaliar os efeitos dos fertilizantes orgânicos e minerais no sequestro de C. Os resultados destes estudos podem trazer valiosa contribuição tanto na tomada de decisão quanto na escolha do sistema de produção a ser adotado por agricultores, como também na destinação correta aos dejetos e a manutenção dos estoques de C e N, auxiliando na mitigação do efeito estufa. Dentro do exposto, este trabalho apresenta os seguintes objetivos:

I) Determinar os estoques de C e N no solo em sistema integrado de produção agropecuária (iLP e iLPF), utilizando-se adubação orgânica e inorgânico, bem como o aporte de C e N pelas culturas e o sequestro de C nos dois sistemas.

II) Caracterizar os teores de C e N e a diversidade da comunidade microbiana em sistemas integrados de produção agropecuária (iLP e iLPF) após cinco anos de cultivo.

CAPÍTULO 1

Soil carbon and nitrogen stock in two tropical integrated systems using organic and inorganic fertilizers

Abstract

Integrated production systems with organic and inorganic fertilizers are promising alternatives for sustainable agriculture, allowing positive interactions between animals and plants, which results in environmental benefits and economic viability. The objective of this study was to investigate the C and N stocks on soil from two integrated production systems (crop-livestock - ICL and crop-livestock-forest - ICLF), from a crop-livestock-forest integration system, implemented in 2015 in southwest Brazil. Soil received organic and inorganic fertilization and samples were collected in 2019 and characterized by physical-chemical parameters, and to C and N content measurement. The results showed, at the 10 – 20 cm layer, the highest C stocks were found in the poultry litter in the ICL system, and all treatments showed higher N stock than the control treatment in this production system. Higher C stocks were observed until 40 cm depth in the ICL system for poultry litter and liquid swine manure treatments. N stocks were higher in the first 10 cm soil layer, where the poultry litter treatment showed higher stocks in the ICL system. Poultry litter and liquid swine manure treatments increased N input in soil, which is an advantage of using these fertilizers over inorganic fertilizers. The ICLF system is superior to the ICL for C input when there is no fertilization, whereas the ICL system is superior to ICLF for N input when there is fertilization with poultry litter and inorganic. Fertilization with either inorganic or organic fertilizers increased the C input only in the ICL system.

1.1 Introduction

Approximately 80% of Earth carbon is stocked in the soil (Lal 2014). Intensive agricultural practices have occasioned high soil degradation, causing negative impacts on carbon stocks, mainly by altering natural ecosystems to convert them into other types of agricultural systems (Calonego et al. 2012; Lacerda et al. 2013).

The restoration of degraded soils is one of the most effective actions that can be taken to promote greater carbon accumulation, through applying conservationist cultivation practices, performing crop rotation, improving pastures, and planting of specific tree species (Lal 2003). Thus, the management adopted may have direct implications for the maintenance of the chemical, physical and biological attributes of the soil, which may then result in large variations in carbon stocks (Parron et al. 2015; Primieri et al. 2017).

Conservationist soil management practices have the potential to maintain soil quality and high levels of carbon sequestration (Sá et al. 2017; Six et al. 2004). In view of the need to recover agricultural soils and increase carbon stocks, integrated agricultural production systems such as crop-livestock-forest (ICLF) reveal themselves as excellent alternatives, due to their ability to improve soil quality and carbon storage (Sarto et al. 2020a).

Fertilization with organic residues can promote an increase in the organic matter content of the soil, reflecting positively on the total organic carbon content (Yang et al. 2015), this is especially true for liquid swine manure and poultry litter organic fertilizers (Mafra et al. 2015; Hentz et al. 2015). These organic residues have high levels of nutrients that are essential for plants (Zavattaro et al. 2017) and their positive balance is essential for increasing soil organic matter (Nunes et al. 2011).

The assessment of soil carbon and nitrogen levels in different integrated product systems after the application of organic residues may suggest new measures and indicate the appropriate management to stock these nutrients. Therefore, the objective of this study was to investigate the C and N stocks in two integrated production systems (ICL and ICLF), using organic and inorganic fertilization.

1.2 Materials and Methods

Site description

Samples were collected from both integration system, crop-livestock and crop-livestock-forest, implemented in 2015 in south Brazil, in Concórdia city, state of Santa Catarina (52°04′ 58,22” W, 27° 12′ 0,08” S). As the coldest months (June and July) scored average temperatures of around 15°C and the average annual temperatures scored around 23°C, and its total annual rainfall was above 1,500 mm, this region was classified as Cfa (Cfa = Humid subtropical climate) according to the Köppen climate classification system. Meteorological data is provided in Fig. 1.

The soil chemical and physical attributes of soil were analyzed according to the methodology proposed by Embrapa (2007). Characteristics were determined (0–20 cm) before the experiment, in 2014 (Table 1). The soil was characterized as Rhodic Kandiudox (IUSS Working Group WRB, 2015).

Figure 1 – Precipitation and temperature variation in the experimental area in 2015-2019

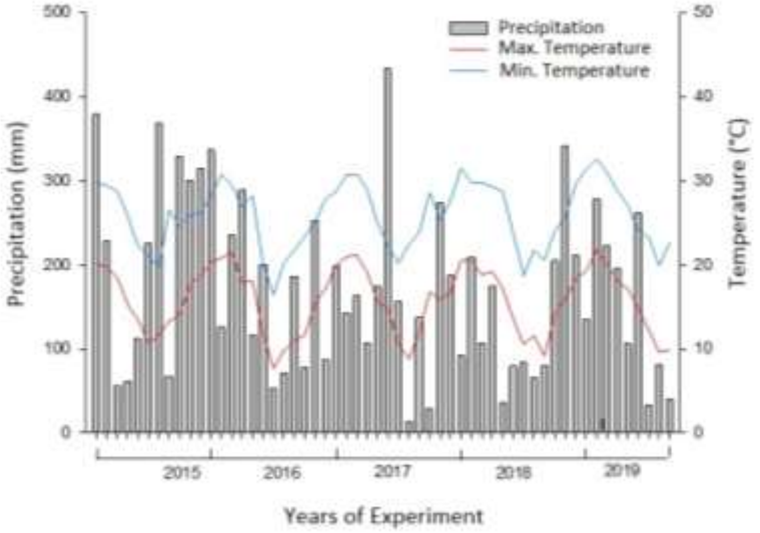


Table 1 – Physical and chemical properties of Rhodic Kandiudox in the study area in 2014

Soil characteristics	Layer		
	0.00 – 0.05 m	0.05 – 0.10 m	0.10 – 0.20 m
Density (g/cm^3)	1.10	1.33	1.45
Soil pH	5.8	5.6	5.5
Clay (g kg^{-1})	680	680	700
N (g kg^{-1})	1.9	1.7	1.5
Cu (mg kg^{-1})	4.7	5.5	4.4
K (mg kg^{-1})	590	406	346
Mg (cmol$_c$ kg^{-1})	4.8	4.0	4.2
Ca (cmol$_c$ kg^{-1})	8.4	6.7	9.5
H + Al (cmol$_c$ kg^{-1})	5.7	6,0	5.8
[a]CEC (cmol$_c$ kg^{-1})	20.5	17.8	20.5
[b]BS (%)	72	66	72
P (mg kg^{-1})	100	80	70
Zn (mg kg^{-1})	5.1	4.4	3.6
COT (g kg^{-1})	18	17	17

[a] CEC: cation exchange capacity.
[b] BS: Base saturation

Experimental design and treatments

A 2 × 4 factorial experimental design was randomly applied, with three replications. The treatments included two systems of conservationist production: Integrated crop-livestock (ICL) and Integrated crop-livestock-forest (ICLF) (Fig. 2) and interaction with three different fertilizers (liquid swine manure, poultry litter and inorganic). The control treatment area did not receive any fertilizer.

Figure 2 – Integrated crop-livestock-forest system (A) and Integrated crop-livestock system (B)

Site history

The experiment was conducted in an integrated agricultural production system (no-till system) installed in 2015. The *Eucalyptus* (*Eucalyptus dunnii*) were planted east-west at a spacing of 2.0 m x 2.0 m between plants. The integrated crop-livestock-forest (ICLF) was made up of two eucalyptus rows and a 30 m spacing between each row. The resulting density was of 300 plants ha^{-1}. Each plot contained two rows of eucalyptus.

The summer crops during 2018 and 2019 were maize from the Pioneer (30F53VYHR) company. Sowing was carried out on December 15, 2017, and on December 11, 2018. The spacing used was of 0.70 m with a population of 60,000 plants per hectare. The seeds were placed at a depth of 5 cm. Harvesting took place on April 2018 and on April 2019. Winter crops in 2018 were wheat (BRS Taruma / Embrapa), sown in May at a density of 70 kg ha^{-1} with a spacing of 30 cm and a depth of 5 cm. In 2019, black oats (BRS 139 / Embrapa) were sown on May at a density of

80 kg ha^{-1} with a spacing of 30 cm and depth of 5 cm, used for grazing and hay. During the winter period, beef cattle were grazed with subsequent biomass sampling after the last grazing to characterize the C and N input in the system for these crops in October 2018 and in October 2019.

The fertilizer treatments consisted of poultry litter, liquid swine manure and inorganic, applied according to the recommended techniques for each crop at a certain time of the year (summer and winter), from the critical limit of P (Table 2). Fertilizer application was carried out on the surface in the total area and in the eucalyptus crop next to the plant line. Liquid swine manure was produced on a complete cycle breeding system, where animals remained housed in a compact floor system from birth to slaughter with an average live weight of 120 kg and an average age of 145 days. Liquid swine manure was then stocked in digestion lagoons for 120 days to stabilize until its use as organic fertilizer. Poultry litter used in this research came from at least six different poultry flocks. The analysis of organic fertilizers was carried out according to official analysis recommendations (AOAC International 2000 and APHA 1992).

Table 2 – Nutritional report of organic fertilizers in the integrated production system

	Nutrient content in fertilizer (g kg^{-1} or L^{-1})			Dose 100%	P input	N input	C input
	N	P	C	(kg or L ha^{-1})		(kg ha^{-1})	
Soybean cultivation 2015/2016							
Poultry litter	22.1	10.6	282	2,262	24	50	638
Liquid swine manure	3.4	0.9	10.5	14,706	13	50	154
Corn cultivation 2016/2017							
Poultry litter	25.5	15.3	296	3,921	60	100	1,161
Liquid swine manure	2.5	0.35	5.7	40,000	14	100	228
Corn cultivation 2017/2018							
Poultry litter	24.2	12.6	263.1	4,132	52	100	1087
Liquid swine manure	2.7	0.9	9.8	37,037	33	100	363
Corn cultivation 2018/2019							
Poultry litter	16.2	5.3	280	6,172	33	100	1,728
Liquid swine manure	3.3	1.0	19.3	30,303	30	100	584
Sum of input during system conduction							
Poultry litter				16,487	169	350	4,614
Liquid swine manure				122,046	90	350	1329

Measurement of C and N stocks in the soil

Soil samples from the 0–10, 10–20, 20–40, 40–60, 60–80 and 80–100 cm depths (3 samples per plot) were collected in the open prefills in September 2019, to determine stock organic carbon (SOC) and total nitrogen (TN). The collection was performed with a shovel, and only the central part of the sample was used. To determine soil density, undisturbed samples were collected with rings.

The samples were air-dried, poured through a 2 mm sieve, ground with a pestle and then passed through a 500 µm sieve, removing all plant material. To determine the total content of C and N, an elementary analyzer (Model CNHOS Thermo Fisher Scientific / Flash 2000 / Cambridge / UK) was used, applying the dry combustion method (900 °C).

C and N stocks were quantified using the equivalent soil mass method (ESM), which compares the concentration of C and N, the layer thickness, the soil density and the equivalent soil mass of the soil undergoing the experiment treatments to those of the soil with control treatments (soil before experimentation) (Wendt and Hauser, 2013).

Dry biomass of crops and C and N content

The plant residues of the 2018 and 2019 summer and winter periods were sampled to determine the dry mass of the cultures. Random sampling was performed at four points of each treatment with the aid of a model (0.25 m^2), while the roots were estimated at 25% of the biomass of the aerial part, according to the methodology proposed by Correa et al. (2019). Winter plants were collected after animal grazing, cutting them 1 cm above the ground, whereas summer plants were collected after harvesting. The material was packed in paper bags, dried in an oven with forced air circulation at 65 °C until it reached constant mass, and then weighed. For C-total and N-total analysis, 15 mg subsamples were collected and determined using an automatic elementary analyzer (Flash EA 1112 Series Thermo Finnigan Italia S.p.A., MI, Italy). The samples were collected in September 2018 and 2019. The diameter at breast height (DBH - 1.30 m above the ground) of ten plants per plot was evaluated in September 2018 and 2019. A Haglof Electronic Clinometer was used to measure the total height of the tree. The total eucalyptus biomass (m^3) was obtained by multiplying the stem volume by the specific density of the eucalyptus wood, which product was then multiplied by 1.6, assuming that a unit of biomass from the stem wood is associated with 1.6 units of biomass from the entire tree, including the stem, branches and leaves (Dixon et al. 1993). The accumulation of C in the trees was assumed to be 50% of the total biomass (Gifford 2000; IPCC 2007; Penman et al. 2003).

Statistical analysis

To calculate the concentrations and stocks of C and N in the ICLF experimental units, the weight average of the concentrations was calculated considering the proportion of 0.92 for the crop area and 0.08 for the *Eucalyptus* area. After calculating the concentrations per experimental unit, analysis of variance was performed for the randomized block design model. In the plots, the effect of the production system was evaluated, and in the subplots, the effects of fertilization and its interaction with the production system. To detail the fertilization effect within the combination of the production system and layer levels, the protected t-test was applied, whenever the F test detected a significant effect ($p \leq 0.05$). The analysis was performed for each soil layer independently. The MIXED procedure of the statistical software SAS (2012) was used to perform the analysis.

1.3 Results

Carbon and nitrogen input in the system through crop biomass

After 5 years of ICL and ICLF systems implementation, fertilized with organic and inorganic fertilizers, it is possible to verify an increase in the C input in both systems in 2018, although it was significant only among fertilizers in 2019 (Table 3). The highest C inputs can be observed when the fertilization recommendation is made, with swine manure and poultry litter being the most efficient treatments in both production systems (ICL and ICLF) in 2018; the poultry litter repeated the same performance in 2019, and the inorganic treatment presented the highest value (4093 kg ha^{-1}) only in the ICL system.

When there is no application of fertilizers, there was significant difference, as observed in 2018, where the control treatment displayed greater efficiency in the C input in the ICL system (2,590 kg ha^{-1}) than in the ICLF system (2,030 kg ha^{-1}); no other differences were found when comparing production systems.

Table 3 – Input of C (kg ha^{-1}) and N (kg ha^{-1}) by the crops and *Eucalyptus* in different years of evaluation and respective standard errors according to the production and fertilization system.

Production System	Fertilizers			
	Poultry litter	Inorganic	Liquid swine manure	Control
C Input Crops 2018 (kg ha^{-1})				
ICL	3234 ± 188 ^{a}	2531± 188 ^{b}	3399± 188 ^{a}	2590 ± 188 ^{bA}
ICLF	3670 ± 188 ^{a}	3013± 188 ^{b}	3552± 188 ^{a}	2030 ± 188 ^{cB}
C Input Crops 2019 (kg ha^{-1})				
ICL	3953 ± 118 ^{ab}	4093± 118 ^{a}	3652 ± 118 ^{bc}	3413 ± 118 ^{c}
ICLF	4218 ± 118 ^{a}	3836± 118 ^{b}	3663 ± 118 ^{b}	3208 ± 118 ^{c}
N Input Crops 2018 (kg ha^{-1})				
ICL	181.46±8.56 ^{a}	126.69±8.56 ^{b}	177.88±8.56 ^{a}	113.84±8.56 ^{b}
ICLF	199.95±8.56 ^{a}	147.66±8.56 ^{b}	184.34±8.56 ^{a}	93.51±8.56 ^{c}
N Input Crops 2019 (kg ha^{-1})				
ICL	133.58±4.58 ^{ab}	141.03±4.58 ^{a}	121.11±4.58 ^{b}	121.20±4.58 ^{b}
ICLF	139.91±4.58 ^{a}	128.38±4.58 ^{ab}	122.40±4.58 ^{bc}	113.11±4.58 ^{c}
C *Eucalyptus* 2019 (Mg ha^{-1})				
ICLF	27.22±1.56	24.19±1.56	29.59±1.56	30.81±1.56

Means followed by distinct lower-case letters on different lines differ by protected t-test (p ≤ 0.05); Means followed by different capital letters in the different columns differ by the F test (p ≤ 0.05).

The N input by the crops followed the same results obtained by the C input, where the poultry litter and swine manure fertilizers were more efficient in 2018 in both systems; the poultry litter continued the same performance in 2019, being similar to the inorganic treatment, also with significant difference in both production systems (ICL and ICLF). For this variable, there was no difference among production systems when no fertilizer was applied in both years.

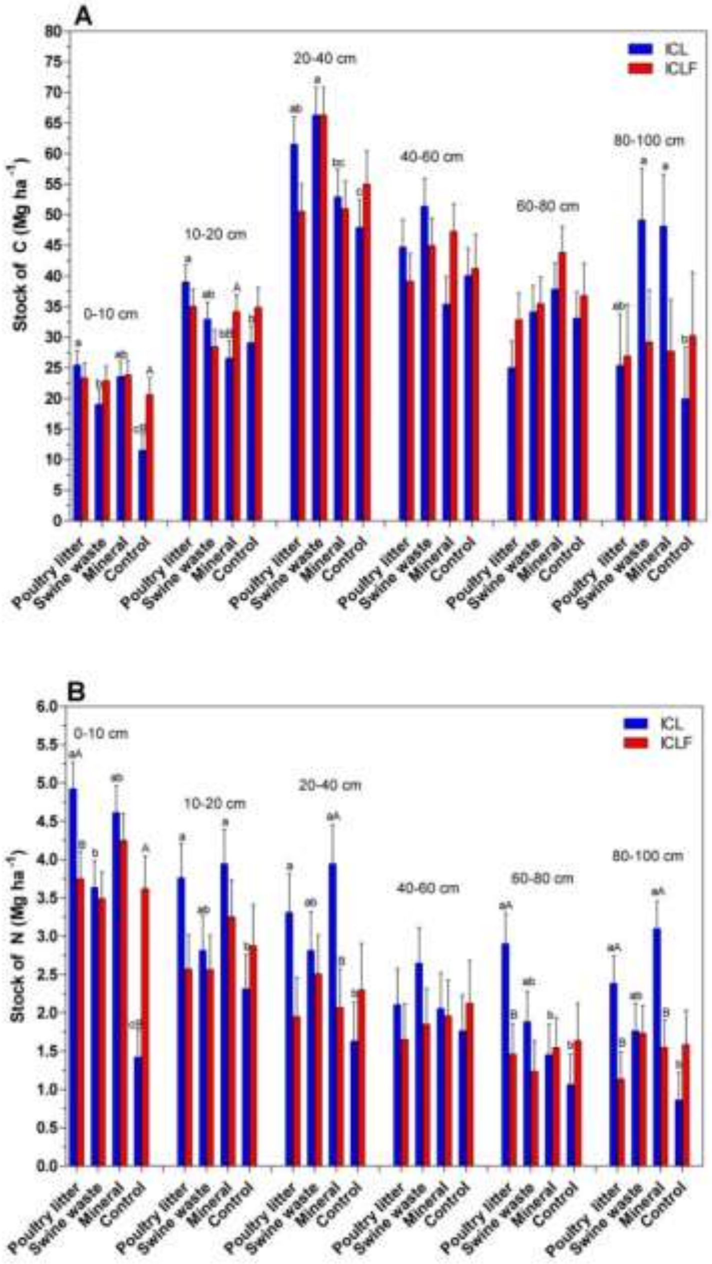
The eucalyptus cultivation present in the ICLF system, although measured over the years, only the latter was presented to characterize the sum of its biomass (aerial part and roots) that were transformed into C and N inputs. This criterion demonstrates the total amount the system has at that moment, therefore leading to C sequestration discussion. It is worth mentioning that there is no significant difference between treatments with and without fertilizers.

Carbon and nitrogen stock in soil layers

The stock of C and N (Mg ha^{-1}) equivalent mass in the soil layers and respective standard errors varied according to the production system and fertilization (Figure 3). Among the production systems, significant differences were observed for C stock in the 0–10 cm layer with control treatment, and in the 10–20 cm layer with inorganic treatment, both in the ICLF system. With respect to N stock, differences were found in the 0–10 cm layer in ICL system using poultry litter and in ICLF system for the control treatment; and for the 80–100 cm layer, both the poultry litter and the inorganic treatments showed greater N stock in ICL system.

The fertilizers only influenced C and N stocks in the ICL system. The highest C stocks occurred for poultry litter in layers 0–10, 10–20, 20–40 cm; for inorganic in layers 0–10, and for swine manure in the layer of 20–40 cm (Figure 3). The recommended treatment for raising the C stock, in the 0–10 cm layer, is the poultry litter, which shows an efficiency of around 150%. The largest N stocks were found in the poultry litter along the soil profile up to a depth of 100 cm, with the exception of only the 40–60 cm layer, in which the inorganic treatment was similar, up to the depth of 40 cm, and later in the 80–100cm layer, while swine manure showed this same efficiency in 10–20, 20–40 and 80–100 cm layers.

Figure 3 – Least squared adjusted means for the C and N stocks (Mg ha^{-1}) equivalent mass in the soil layers and respective standard errors according to the production and fertilization system



Carbon and nitrogen stocks in the soil profile

In the total soil profile, there is a difference for C stock among production systems only in the control treatment, with ICLF being higher than ICL in the order of

20.7% (Table 4). Regarding N stock, the difference among production systems occurs in poultry and inorganic treatments, the ICL being superior to the ICLF.

Table 4 – Least squared means for C and N stocks in the 0 to 100 cm layer of soil and respective standard errors according to production and fertilization system

Production System	Fertilizers				
	Poultry litter	Inorganic	Liquid swine manure	Control	Mean
			Stock_C_Equ_Mass (Mg ha^{-1})		
ICL	220.99±12.2^{bc}	224.36±12.2^{a}	252.82±12.2 ^{a}	181.61±12.2^{bB}	219.95±7.88
ICLF	207.82±12.2	227.78±12.2	227.19±12.2	219.23±14.6 ^{A}	220.51±8.13
			Stoch_N_Equ_Mass (Mg ha^{-1})		
ICL	29.60±2.67^{aA}	29.43±2.67^{aA}	24.45±2.67 ^{a}	14.64±2.67 ^{b}	24.53±1.90
ICLF	18.09±2.67 ^{B}	21.04±2.67 ^{B}	20.36±2.67	21.13±3.13	20.15±1.94

Means followed by distinct lower-case letters on different lines differ by protected t-test ($p \leq 0.05$); Means followed by different capital letters in the different columns differ by the F test ($p \leq 0.05$).

The difference among the fertilizers regarding C stocks only varied in the ICL system, with 252.82 Mg ha^{-1} for liquid swine manure and 224.36 Mg ha^{-1} for inorganic, with the former displaying the highest difference from its control treatment counterpart (an increase of 71.21 Mg ha^{-1}). The ICL system yields a greater N input than its control treatment counterpart with organic and inorganic fertilizers, with an efficiency of 102.2%. There is no difference between these fertilizers in the ICLF system.

Carbon sequestration

ICL values of C sequestration for the poultry litter, liquid swine manure, inorganic and control treatment were of 228.1, 260, 231.5 and 188.7 Mg ha^{-1}, respectively. ICLF values were 242.9, 264.6, 262.8 and 257.9 Mg ha^{-1}.

The presence of eucalyptus in the ICLF system caused increases of 14.7, 4.6, 31.3 and 69.1 Mg ha^{-1} for the poultry litter, liquid swine manure, inorganic and control treatments. It also represented an increase of 30.9 Mg ha^{-1} between systems.

1.4 Discussion

Carbon and nitrogen input in the system through crop biomass

In relation to fertilizers, the largest inputs of C and N in 2018 were observed in poultry litter and liquid swine manure, with an efficiency of 56% for C in the ICL system and 59% in the ICLF system; the efficiency for N was 60% in the ICL system and 62% in the ICLF system. In 2019, C and N inputs had an efficiency of 27% for the inorganic fertilizer in the ICL and poultry litter in the ICLF system.

The dynamics of C in the soil can be changed according to the history of the system management (Romanenkov et al. 2019). High fertility soils do not require much fertilization in order to yield large inputs of C. This is especially true for soils with grasses (Sant-anna et al. 2017). Nonetheless, fertilization improves soil fertility, and over time, less fertilizer is required to obtain the same inputs of C.

Agricultural crops have greater input of C in ICL than in ICLF systems (Bieluczyk et al. 2020). The same holds true for pasture (Sarto et al. 2020b). These results are related to the insertion of the forest component in the ICLF system, which reduces the agricultural crop area and photosynthetic efficiency due to shadows cast by the trees (Pezzopane et al. 2020). Nevertheless, the presence of trees promotes greater C and N inputs, which must be added to the values from annual crops and pastures in ICLF systems.

Thus, for the maintenance of C stocks in the soil, the correct choice of cover plants is essential, in addition to adequate cultivation time (Soares et al. 2020). Corroborating the data for 2019, Liu et al. (2020) did not observe different values of C inputs between systems. However, the same authors emphasize the importance of C input through the addition of residues for the rotation of crops with wheat.

The fact that the poultry litter and liquid swine manure treatments increased N input on the soil presents a great advantage on the use of these fertilizers over inorganic fertilizers, due to the residual effect of the nutrient (De Resende et al. 2019; Yang et al. 2015). Inorganic fertilizers present greater N losses through leaching and volatilization (Raij et al. 2007). Thus, the results presented suggest that organic fertilization with residues can promote greater accumulation of biomass in the cultures of both systems, promoting greater inputs of C and N in the soil.

Carbon and nitrogen stock in soil layers

Among the great contributions of this research to generate knowledge between the ICL and ICLF production systems is the result of stocking C at a depth of 0–10 cm, which showed the superiority of the ICLF system when fertilization does not occur, being this efficiency demonstrated in order of 78%. These findings corroborate those found by Tonucci et al. 2011, showing a larger C stock under monoculture pasture than under silvopasture in the Brazilian Cerrado. Carbon sequestration of 3.0 to 14.0 Mg C ha $^{-1}$ year $^{-1}$ for grasses, however, 75% of the sequestered C was below 0.20 m (Fisher et al. 1994).

Don et al. 2011; Thomazini et al. 2015, observed that the soil carbon stocks can be used to analyze sustainability and indicate the proper management in agricultural production systems

However, C stock variations can occur due to the chemical characteristics of the soil (Table 1), as well as to the contribution of plant residues caused by successive cultivation. According to West and Post (2002), a 5 to 10-year period of crop rotation is important to preserve the soil carbon sequestration capacity. In this scenario, the dynamics of C in the soil was changed due to the cultivation history of the area (Romanenkov et al. 2019).

In the study published by Wuaden et al. (2020), a greater accumulation of N was observed in the superficial layer of the soil (0 – 30 cm) in no-tillage systems with organic fertilizers, thus avoiding N losses. According to the authors, this accumulation in the superficial layer happens due to the presence of superficial roots.

Carbon and nitrogen stock in the soil profile

As the ICLF system did not show any difference between treatments, with and without fertilization, it is possible to infer about the cycling capacity of these nutrients assigned to the eucalyptus roots and also due to the lower temperature variation in the soil, conditioning changes in the diversification of the soil microbial community.

De Stefano and Jacobson (2017) found no significant differences in SOC stocks in the soil profile (1.0 m) in a meta-analysis with 53 studies in pasture areas converted to agroforestry. In evaluations carried out by Nair et al. (2010) to estimate C stocks in the soil, values ranging from 0.29 to 15.21 Mg C ha $^{-1}$ year above the ground and 30-

300 Mg C ha $^{-1}$ at a depth of 1 m were found in the soil. Regarding stock assessments of C in Brazilian soils, Batjes (2005) found variations from 42 Mg C ha^{-1} in Quartzenes Neosols to 137 Mg C ha^{-1} in Gleysols at a depth of 1.0 m.

The greater superiority of N stock in poultry litter and manure treatments are justified by fertilizers made with these sources occupy 100% of their area; while at ICLF system, an amount of 92% of the area is used, because 8% is occupied by eucalyptus plantation.

Carbon sequestration

Among the major contributions of the present research is the finding about the ICLF system, which in the condition that fertilization does not occur, increases the C sequestration by 69.1 Mg C ha^{-1}, a result that justifies implementing this system for greater sustainability with regard to mitigating greenhouse gases, nutrient cycling and greater diversification and population of the microbial community. Sarto et al. (2020a) found values of 44.3 Mg C ha^{-1} above ground in the ICLF system and 14.1 Mg C ha^{-1} in the ICL system in a study carried out in the state of Sao Paulo, Brazil, thus demonstrating a better efficiency of systems with the forest component. The ICLF system resulted in higher total C sequestration (above and below ground) values than the ICL system, with an efficiency of 50%. These results match the ones found in this study with an average between the treatments of 30.9 Mg C ha^{-1}.

Due to the higher production of biomass from trees, agroforestry systems have greater potential for C sequestration, compared to conventional production systems. However, further studies of the entire plant cycle are necessary to obtain more effective estimates of the potential for C sequestration in these systems (Sanchez 2000; Roshetko et al. 2002).

1.5 Conclusion

The ICLF system is superior to the ICL for C input when there is no fertilization; whereas the ICL system is superior to ICLF for N input when there is fertilization with poultry litter and inorganic.

Fertilization with either inorganic or organic fertilizers increased the C input only in the ICL system.

The presence of eucalyptus in the ICLF system altered the C and N input values. In this system, fertilization does not influence the numbers to a great extent, which justifies a greater cycling efficiency for both C and N in this system.

Therefore, it can be assumed that, with its higher values of C input and stock, the ICLF system provides greater sequestration, and its use is recommended for greater sustainability and to mitigate climate change.

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CAPÍTULO 2

Soil microbial community in agroforestry systems with organic and inorganic fertilizers

Abstract

Integrated production systems, such as agroforestry, are promising alternatives for sustainable agriculture, allowing positive interactions between animals and plants, which results in environmental benefits and economic viability. Soil health is associated to C and N stocks, and the microbial community composition. Therefore, the present study aimed to characterize the soil C and N stocks and the microbial community profile in a tropical Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forest (ICLF) systems after five years of cultivation, applying poultry litter, liquid swine manure and inorganic fertilizer. Soil samples were collected from an integrated crop livestock-forest to C and N contend measurement, and microbial community diversity determination. The results showed that C stocks increased along soil depth and N stocks are higher in the soil first 10 cm layer, as well as the microbial community concentration, with gram-negative bacteria been the majority fraction. In the ICLF system, no statistical difference was found among the applied fertilizer. In the ICL system, the use of poultry litter fertilizer showed significantly higher N stocks in the soil. The results emphasize that agroforestry systems benefit agriculture in a sustainable way.

Keywords: Nutrient stocks, Bacteria, Fungi, *Eucalyptus dunnii*

2.1 Introduction

The modern agriculture aims to find the equilibrium between production and environmental conservation. In this sense, integrated production systems such as agroforestry, are promising alternatives for sustainable agriculture, allowing positive interactions between animals and plants, which result in environmental benefits and economic viability (Balbinot Junior et al., 2009), once agroforestry can improve soil conservation, CO_2 sequestration and nutrient availability (Beule et al., 2019). Other systems, such as crop rotation and the use of cover crops, along with no-tillage, are agricultural practices that allow soil preservation over time (Coser et al., 2018; Lemaire et al., 2015).

The use of these systems can change the dynamics of soil carbon (C) stock, which can influence the quantity and quality of the soil organic matter (SOM) and the microbial community composition (Sarto et al. 2020a). Thus, C stock can be used to evaluate the ideal management of agricultural systems (Don et al., 2011; Thomazini et al., 2015).

Soil nitrogen stocks can be changed by cultivating plant species, such as leguminous plants (Aita et al., 2001; Da Ros and Aita, 1996; Fugita et al., 1992; Rao and Mathuva, 2000), but also by the application of organic material as fertilizer, which are rich in nitrogen (Peoples et al., 1995). In this sense, the use of fertilizers can significantly change soil properties, microbial activity and the composition of the microbial community in integrated production systems (Gu et al., 2017; Li et al., 2014; Mandal et al., 2007).

The knowledge about soil dynamics during different management conditions is essential to support the production system choice to promote environment protection and crop productivity quality. Therefore, the present research aimed to characterize C and N stocks of the soil and the microbial community profile in a tropical integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forest (ICLF) systems after five years of cultivation.

2.2 Materials and methods

Site description

Soil samples were collected from an integrated crop livestock-forest system, implemented in 2015 in southwest Brazil, in the city of Concórdia, Santa Catarina (52°04′ 58,22” W, 27° 12′ 0,08” S). The local climate in this region has an average temperature of 15 C and 23 C respectively in the coldest (June-July) and warmest (December-January) month. The annual total rainfall was above 1,500 mm (Wrege et al., 2012). The soil was classified as Rhodic Kandiudox (IUSS Working Group WRB, 2015).

Soil cultivation history

The soil samples were collected in two integrated systems: Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forest (ICLF), both installed in 2015. Table 1 shows the culture history from both systems during five years prior to soil sampling events. In the ICLF system, eucalyptus (*Eucalyptus dunnii*) was planted at a spacing of 2.0 m x 2.0 m, composed by two eucalyptus rows and 30 m spacing between each row, resulting in a density of 300 plants ha^{-1}. The cattle (crossbred) were introduced in 2016 and raised until slaughter weight in a continuous grazing system with 2.0 animal unit ha^{-1}.

Table 1 – Culture history from Integrated Crop-Livestock and Integrated Crop-Livestock-Forest

Year	Culture	Description
Summer 2015 to 2019	Maize (*Zea mays*)	spacing of 0.70 m with a population of 60,000 plants per hectare. The seeds were placed at a depth of 5 cm. Harvested after four months.
Winter 2016 to 2018	Wheat (*Triticum aestivum*)	density of 70 kg ha^{-1} with a spacing of 30 cm and 5 cm depth. Beef cattle were grazed.
Winter 2019	Black oats (*Avena strigosa)*	density of 80 kg ha^{-1} with a spacing of 30 cm and 5 cm depth, used for grazing and hay. Beef cattle were grazed.

Soil fertilizer application

To evaluate the influence of fertilizer materials, both ICL and ICLF systems received addition of different fertilizer materials: poultry litter, liquid swine manure, as inorganic fertilizer, all applied according to the agronomical techniques for each cultivation. Fertilizer application was carried out on the surface in the total area, and in the eucalyptus crop inorganic fertilizer were applied next to the plant line. Fertilizer characteristics and application events are presented in Table 2. The chemical characteristics from the fertilizer material were determined following the APHA (1992) methodology.

Table 2 – Characterization of organic fertilizers applied in the integrated production system

	Nutrient content in fertilizer (g kg^{-1} or L^{-1})			Dose 100%	P input	N input	C input
	N	P	C	Kg or L ha^{-1}	(kg ha^{-1})	(kg ha^{-1})	(kg ha^{-}
Soybean cultivation 2015/2016							
Poultry litter	22.1	10.6	282	2,262	24	50	638
Liquid swine manure	3.4	0.9	10.5	14,706	13	50	154
Corn cultivation 2016/2017							
Poultry litter	25.5	15.3	296	3,921	60	100	1,161
Liquid swine manure	2.5	0.35	5.7	40,000	14	100	228
Corn cultivation 2017/2018							
Poultry litter	24.2	12.6	263.1	4,132	52	100	1,087
Liquid swine manure	2.7	0.9	9.8	37,037	33	100	363
Corn cultivation 2018/2019							
Poultry litter	16.2	5,3	280	6,172	33	100	1,728
Liquid swine manure	3.3	1.0	19.3	30,303	30	100	584
Sum of input during system conduction							
Poultry litter				16,487	169	350	4,614
Liquid swine manure				122,046	90	350	1,329

Experimental design and soil sampling

A completely randomized design composed of a 2 × 4 factorial design, with three replications was applied. The treatments included two systems of conservationist production: ICL and ICLF (Fig. 1) and interaction with three different fertilizers (swine manure, poultry litter, inorganic, and control). Areas with the same management characteristics from ICL and ICLF but without fertilizer application were used as controls. Soil samples were collected in September 2019 at depths of 0–10, 10–20 and 20–40 cm. At each depth, three samples per plot to determine soil organic C (SOC) content, total nitrogen (TN) and microbial community characterization.

Figure 1 – Integrated crop-livestock-forest system (A) and Integrated crop-livestock system (B)

Measurement of C and N stocks

The soil samples (500 g) were air-dried, sieved through a 2 mm sieve, ground with a pestle and then passed through a 500 µm sieve, removing all plant material. To determine the total content of C and N, a CN elementary analyzer (Model CNHOS Thermo Fisher Scientific / Flash 2000 / Cambridge / UK.) was used, applying the dry combustion method (900 °C). The C stocks were estimated using the equivalent mass method, taking into account the layer thickness, soil bulk density, the equivalent mass of the reference soil and the soil mass of the treatments, which is taken as the basis for calculating the stock of all other treatments (Bayer et al., 2000; Ellert and Bettany, 1995). The soil mass of the native field layers was considered as a reference, which represents the original soil condition. Soil C and N stocks were estimated according to Sarto et al.(2020b).

Measurement of total microbial biomass

Microbial community was measured by the determination of phospholipid fatty acid (PLFA), because they are only found in viable cells (Tunlid and White, 1992; White et al., 1979). PLFA were extracted from 5 g of each lyophilized soil sample, following White and Ringelberg, (1998). The PLFA extraction was performed with a chloroform: methanol: phosphate buffer solution for 2 h (Bligh and Dyer, 1959), and analyzed by gas chromatography (GC) (HP 6890, Agilent Incorporated, Palo Alto, CA, USA). A 25–m Ultra–2 (J & W Scientific, Agilent Technologies, Palo Alto, CA, USA) column was used with He as the carrier gas at 1 mL min^{-1}. The GC analyzed a 1 μL splitless injection where the inlet temperature was 230 °C, the Gas Chromatography with a Mass Spectrometry Detector (GC-MSD) interface was 280 °C, and the column used was an Agilent Ultra–2 (cross linked 5% PH ME), 25 cm length, 0.2 mm i.d. and 33 μmol L^{-1} film thickness. The temperature program was set at 80 °C for 1 min, then ramped to 155°C at 20°C min^{-1}, then ramped to 270 °C at 5°C min^{-1} and held for 5 min. The total run time was 33 min. Sample peaks were quantified based on comparison of the abundance area of the samples with the area of an internal standard nonadecanoic acid methyl ester (19:0) in terms of nmol g^{-1} dry soil. Fatty acids were grouped into gram-positive bacteria (i15:0, a15:0, 10Me16:0, i17:0, and a17:0), gram-negative bacteria (18:1x7c and cyclic 19:0), actinomycetes (10Me18:0 and 10Me17:0), fungi (18:2ω6,9c and 18:1ω9c), and arbuscular mycorrhizal fungi (AMF) (16:1ω5) (Fabrizzi et al., 2009; McKinley et al., 2005; Pires et al., 2020; Sarto et al., 2020a). The total microbial biomass of PLFA was estimated by determining the sum of all gram-positive and gram-negative bacteria, actinomycetes and fungi.

Statistical analysis

To calculate the concentrations and stocks of C and N and microbial community in the experimental units, the weighted average of the concentrations was calculated considering the proportion of 0.92 for the crop area and 0.08 for the eucalyptus area. After calculating the concentrations (C and N) and amount (microbial community) per experimental unit, analysis of variance was performed for the randomized block design model. In the plots, the effect of the production system was evaluated, and in the subplots the effects of fertilization and its interaction with the production system was evaluated. To detail the fertilization effect within the combination of the production system and layer levels, the protected t-test was applied, whenever the F test detected a significant effect ($p \leq 0.05$). The analysis was performed for each soil layer independently. The MIXED procedure of the statistical software SAS, (2012) was used to perform the analysis. The correlation of soil microbiology and C and N stock concentration was evaluated by applying Pearson correlation using CORR procedure of the SAS software®, (2012).

2.3 Results

Soil C and N stocks

Figure 2 shows the carbon (2A) and nitrogen (2B) stock in different soil depth layers. Considering the ICLF system, there was no statistical difference among the C stock found on soil fertilized with different material in the same layer. When considering the ICL, variation observed on C stock was associated with the applied fertilizer. In general, it is possible to observe that in both studied systems the C stock increases as the soil depth increases (considering the first 40 cm). The profile observed on controls (soil without addition of fertilizer) showed that in the ICLF system, the C stock is significantly higher than the observed in the ICL system.

The results from the N stocks indicate that the highest N stocks are in the first 10 cm soil layer (Figure 2b) in both systems. On soil collected from ICLF system, there was no statistical difference on N stock observed on soil fertilized with different materials. Comparing the first 10 cm soil layer from both systems, the only significant difference between ICL and ICLF observed was in the soil fertilized with poultry litter, with the ICL presenting higher N stocks. The N stocks measured on 20 – 40 cm depth soil layer showed that the concentration present in the soil fertilized with inorganic was significantly higher in the ICL system than the observed in the ICLF system.

Soil microbial community

Figure 3, 4 and 5 shows the microbial community found at each soil layer. In general, it is possible to observe that both systems present similar microbial composition distribution at each soil layer. The highest microbial concentrations are found on the first 10 cm soil layer; and among the microorganism groups, the gram-negative bacteria presented the highest concentrations in all soil layers. The concentration of saprotrophic fungi, which are associated with roots, decreases considerably on soil layer below the 10 cm depth.

Figure 2 – Soil C (A) and N (B) equivalent mass in the soil layers (Mg ha $^{-1}$) observed in Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forest (ICLF) fertilized with different material. Bars depict standard error and different letters denote significant differences ($p \leq 0.05$) according to Tukey test

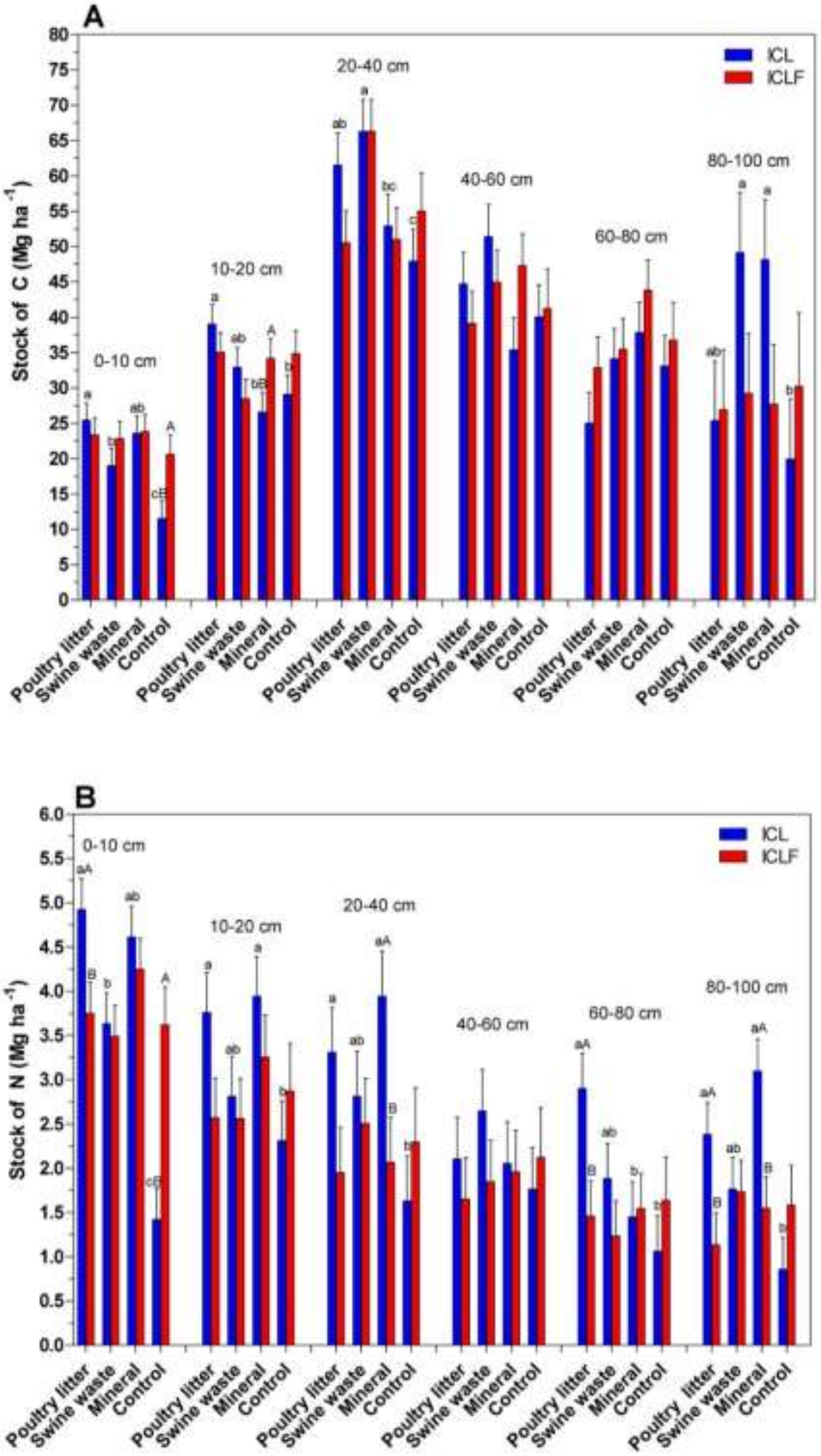


Figure 3 – Soil microbial community observed in Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forest (ICLF) fertilized with different material in the 0 – 10 cm.

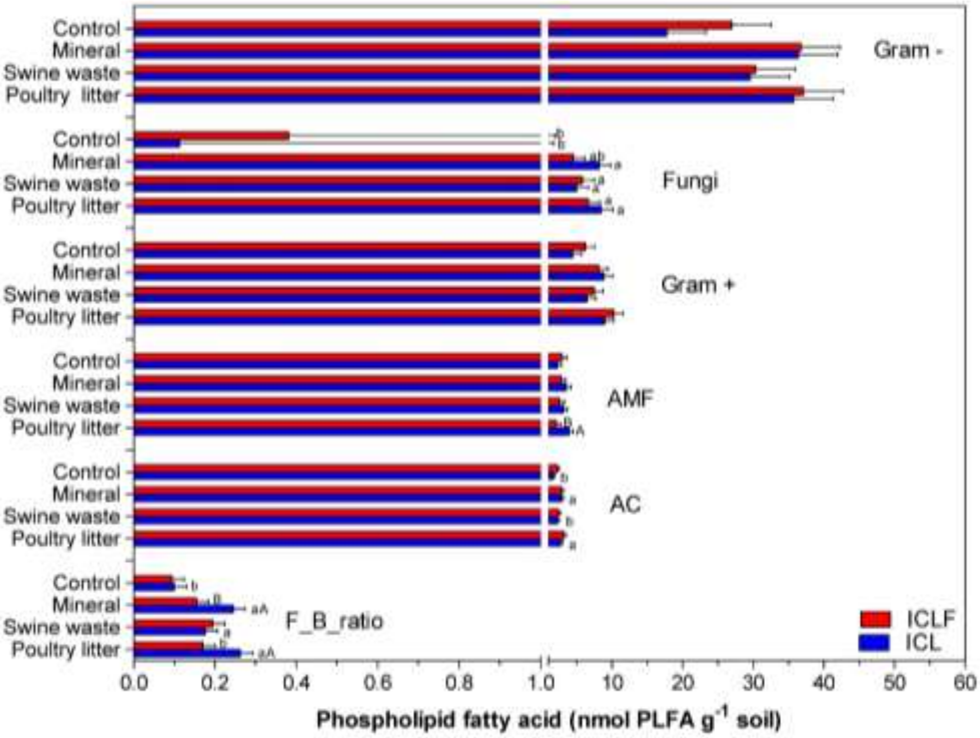


Figure 4 – Soil microbial community observed in Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forest (ICLF) fertilized with different material in the 10 – 20 cm.

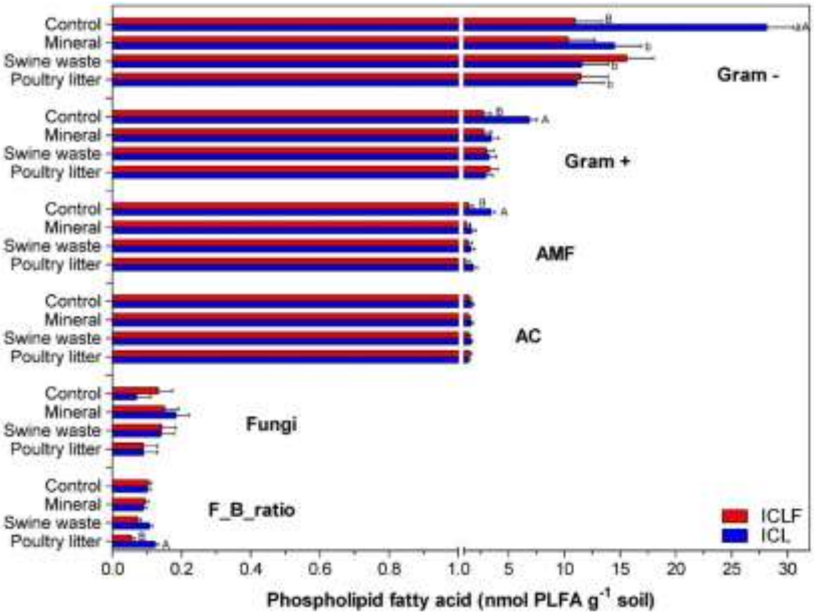
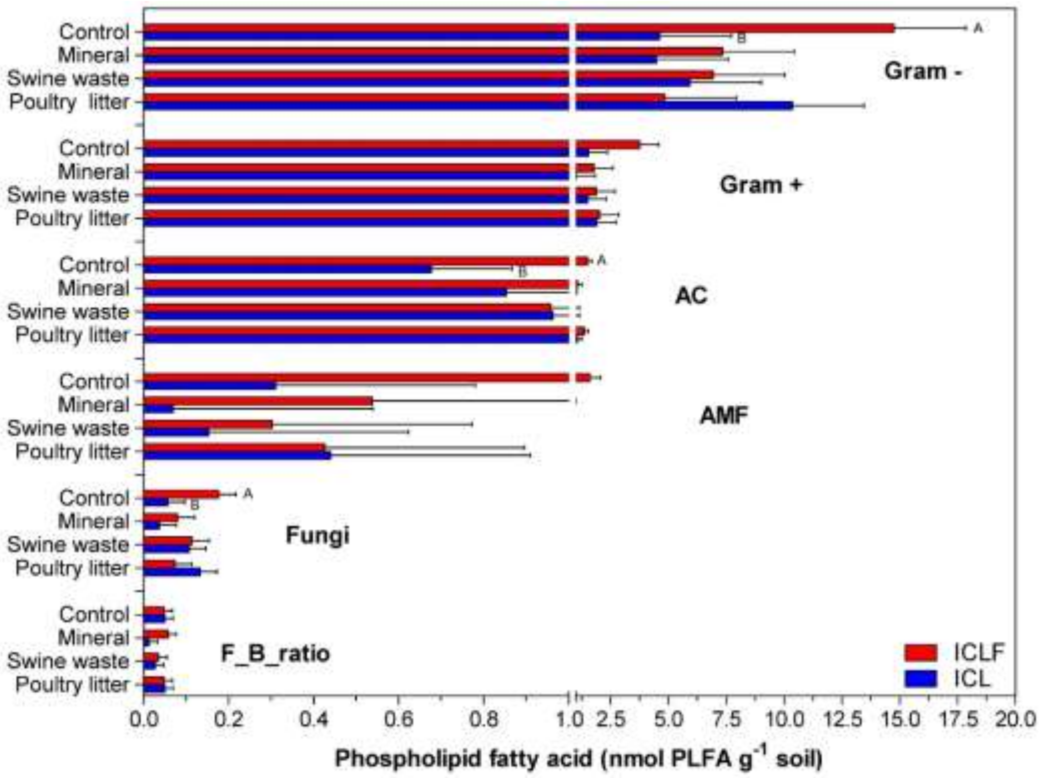


Figure 5 – Soil microbial community observed in Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forest (ICLF) fertilized with different material in the 20 – 40 cm.



2.4 Discussion

The mainly pathway of carbon input on soil can take place by the rhizodeposition (root exudates) that are readily available organic matter, or through slowly process of dead plants decomposition (Jones et al., 2004; Moore et al., 2005, Paterson et al., 2008). Organic fertilizer such as poultry litter or swine manure introduced on soil will be decomposed to CO_2 during organization or will end as part of the organic matter (Cotrufo et al., 2013). The higher C stocks on ICLF when compared to ICL reinforce the importance of these systems when considering the sequestration of CO_2 and transformation on organic C sources (Sarto et al., 2020a). However, the C stocks can vary depending on the soil history, classification, managements and plants cultivated (Dexter, 2004), as observed by Sarto et al., (2020a) which reported that the higher C stocks were present on ICL instead of ICLF. Wuaden et al., (2020) observed higher N stocks on soil surface layer when considering the ICL system, corroborating the present study. These differences on results highlight the importance of studies on different areas to understand the soil dynamics.

The highest microbial concentration in the first soil layer (0 – 10 cm) has consequently the lower C stock since these communities use the C sources to their metabolic activities. The presence of gram-negative bacteria is generally associated with consumption of easily degradable substrates such as recent plant material and root exudates (Creamer et al., 2016), while gram-positive bacteria have been associated with decomposition of soil organic matter composed mainly by dead plants (Kramer and Gleixner, 2006; 2008). Saprotrophic fungi are the responsible for the decomposition of root-derived C (Pausch et al., 2016).

The presence of microorganisms in the first soil layer could be associated to different variables such as higher amount of plant roots, soil aeration, and C availability (Drewry et al., 2008). Geisseler and Scow, (2014) observed that fertilizer application can increase the soil microbial biomass, and consequently the respiration rate, with C consumption.

The soil sampled in the present study was cultivated previously with grass culture, which according to Barber, (1995), can present root densities of 50 cm cm^{-3} (root/soil) in the first soil layer (10 cm) and consequently represent an important source of exudates.

Actinomycetes are aerobic gram-positive bacteria forming filaments, playing important role on organic matter cycling due to the capacity to degrade complex materials from dead plants or hydrocarbons in polluted soils; additionally, they can produce antibiotic substances avoiding plant pathogens in the rhizosphere environment (Bhatti et al., 2017).

Arbuscular mycorrhizal fungi when in symbiosis with plants, improve the decomposition of SOM and nitrogen capture on soil (Hodge et al., 2001; Sharma, 2011). Soil microbial community can be influenced more by soil type rather than management system (Beule et al., 2019).

The results reported in the present study highlight the role of ICLF systems on C stocks increasing on soil, which will be used by microbial community to metabolic activities, increasing the microbial diversity and concentration. Studies have reported that increasing the microbial activity on soil can improve the soil health and consequently improve fertility and plant productivity (Chaparro et al., 2012).

2.5 Conclusion

The results reported here show that ICLF system contributes to increase the soil nutrient stocks and improve the microbial community diversity. In this system there was no difference among the fertilizer material applied, indicating that it is possible to use the fertilizer that is disposable in the region. The ICL system highlight soil characteristics oscillations, demonstrating that the soil is more vulnerable in these conditions. Besides, ICLF improves soil health promoting environmental conservation, and reinforcing the viability of agroforestry systems on sustainable agriculture.

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CONSIDERACÕES FINAIS

A hipótese de que o sistema iLPF pode sequestrar mais carbono em relação ao iLP foi confirmada. A presença do componente arbóreo no sistema representou um acréscimo de 30,0 Mg ha^{-1}.

O uso de fertilizantes não modificou a comunidade microbiana do solo. A maior diversidade de micro-organismos foi encontrada na camada de 20 – 40 cm do solo.

A ausência de adubação resultou em maior aporte de C no sistema iLPF, demonstrando que o componente arbóreo altera a dinâmica de C no solo. No sistema iLP os fertilizantes cama de aves e inorganic resultaram em maiores aportes de N.

O iLPF melhora a saúde do solo, promovendo a conservação ambiental e reforçando a viabilidade dos sistemas agroflorestais na agricultura sustentável.

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