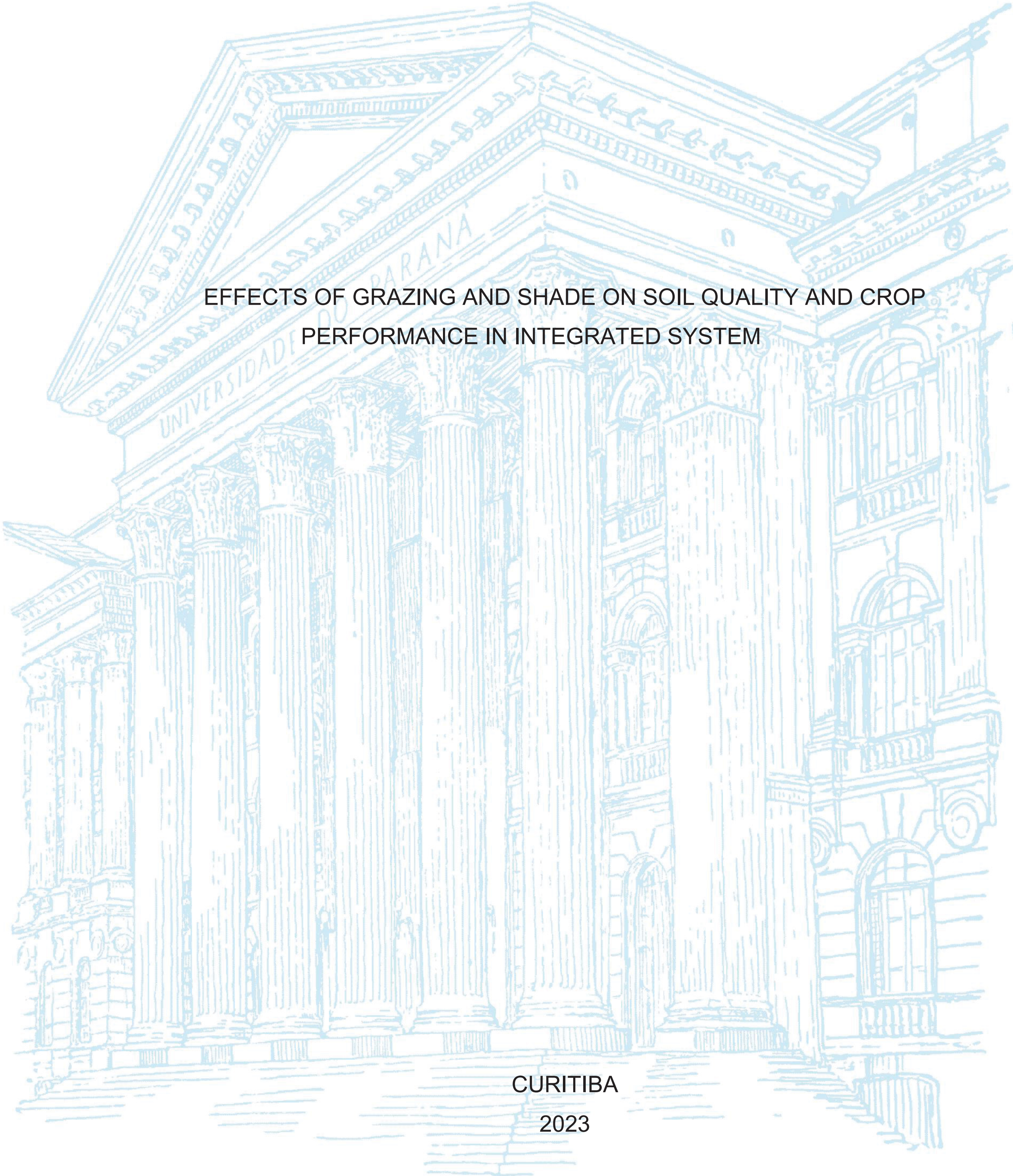
UNIVERSIDADE FEDERAL DO PARANÁ

BARBARA ELIS SANTOS RUTHES



EFFECTS OF GRAZING AND SHADE ON SOIL QUALITY AND CROP PERFORMANCE IN INTEGRATED SYSTEM

CURITIBA

2023

Barbara Elis Santos Ruthes

EFFECTS OF GRAZING AND SHADE ON SOIL QUALITY AND CROP PERFORMANCE IN INTEGRATED SYSTEM

Tese apresentada ao curso de Pós-Graduação em Agronomia, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Agronomia.

Orientador: Prof. Dr. Anibal de Moraes

Comitê de orientação
Prof(a). Dr(a).Glaciela Kaschuk,
Prof. Dr. Leandro Bittencourt de Oliveira
Prof(a). Dr(a). Claudete R. Lang.

CURITIBA

2023

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA DE CIÊNCIAS AGRÁRIAS

Ruthes, Barbara Elis Santos
Effects of grazing and shade on soil quality and crop performance in integrated system/ Barbara Elis Santos Ruthes. – Curitiba, 2023.
1 recurso online: PDF.

Tese (Doutorado) – Universidade Federal do Paraná, Setor de Ciências Agrárias, Programa de Pós-Graduação em Agronomia (Produção Vegetal).
Orientador: Prof. Dr. Anibal de Moraes
Comitê de orientação Profª Drª Glaciela Kaschuk, Prof. Dr. Leandro Bittencourt de Oliveira, Profª Drª Claudete R. Lang

1. Agropecuário - Produtividade. 2. Solos - Qualidade. 3. Agricultura sustentável. I. Moraes, Anibal de. II. Kaschuk, Glaciela. III. Oliveira, Leandro Bittencourt de. IV. Lang, Claudete R. V. Universidade Federal do Paraná. Programa de Pós-Graduação em Agronomia (Produção Vegetal). VI. Título.

Bibliotecária: Telma Terezinha Stresser de Assis CRB-9/944

TERMO DE APROVAÇÃO

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS AGRÁRIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO AGRONOMIA
(PRODUÇÃO VEGETAL) - 40001016031P6

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação AGRONOMIA (PRODUÇÃO VEGETAL) da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **BARBARA ELIS SANTOS RUTHES** intitulada: **EFFECTS OF GRAZING AND SHADE ON SOIL QUALITY AND CROP PERFORMANCE IN INTEGRATED SYSTEM** , sob orientação do Prof. Dr. ANIBAL DE MORAES, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.
A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 21 de Julho de 2023.

Assinatura Eletrônica
25/07/2023 07:58:46.0
ANIBAL DE MORAES
Presidente da Banca Examinadora

Assinatura Eletrônica
24/07/2023 16:02:40.0
GLACIELA KASCHUK
Avaliador Externo (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
25/07/2023 15:59:27.0
LAÉRCIO RICARDO SARTOR
Avaliador Externo (UNIVERSIDADE TECNOLÓGICA FEDERAL DO PARANÁ)

Assinatura Eletrônica
26/07/2023 15:54:16.0
LEANDRO BITTENCOURT DE OLIVEIRA
Coorientador(a)

Dedico este trabalho aos meus pais, cujo esforço incansável tornou tudo isso possível.

AGRADECIMENTOS

Agradeço de forma especial à minha mãe, pois ela foi fundamental ao longo de todo o período de doutorado. Ela é a minha base e a minha força.

Ao meu namorado Luciano Rezende, que não mediu esforços em me apoiar de forma incondicional ao longo de todo esse período.

Ao professor Dr. Anibal de Moraes, expresso minha gratidão por acreditar na capacidade de todos os seus orientandos e por me proporcionar a oportunidade de trilhar este caminho ao seu lado.

Agradeço de coração à professora Dra. Glaciela Kaschuk por toda orientação e conhecimento compartilhados. A senhora foi meu braço forte durante todo o período de doutorado, e sua orientação e amizade foram fundamentais para me tornar mais resiliente ao longo desse percurso.

Gostaria de expressar minha sincera gratidão à Universidade Federal do Paraná e a todos os seus professores e funcionários, cujas contribuições foram essenciais para o desenvolvimento desta tese.

Agradeço especialmente a todos os colegas de pós-graduação, estagiários, acadêmicos e técnicos de laboratório que caminharam ao meu lado, oferecendo valiosos auxílios e contribuindo significativamente para as atividades necessárias à realização deste estudo.

À CAPES pela bolsa de pós-graduação concedida durante todo o período de doutorado.

Everything is temporary, everything will slide

RESUMO

Os Sistemas Integrados de Produção Agropecuária (SIPA) têm despertado um interesse crescente como uma abordagem promissora para diversificar atividades agrícolas e recuperar solos degradados. Esta tese tem como objetivo investigar os efeitos dos ICLS na qualidade do solo, na ciclagem de nutrientes e na produtividade das culturas, a fim de promover uma agricultura mais sustentável. Nossos resultados revelaram melhorias consistentes em diversas propriedades do solo, como a quantidade de carbono da biomassa microbiana (CBM), nitrogênio da biomassa microbiana (NBM), respiração do solo, atividade enzimática, pH, carbono orgânico total (COT) e disponibilidade de nutrientes. Um destaque importante foi o impacto positivo do pastejo animal nos SIPA, que influenciou especialmente as propriedades de biológicas do solo, indicando uma melhor ciclagem de nutrientes e uma melhoria na estrutura do solo. Além disso, a interação entre animais, plantas e a biomassa microbiana do solo nos SIPA mostrou-se benéfica para a nutrição de plantas. A presença de animais nas áreas dos SIPA promoveu uma ciclagem constante de nutrientes, acúmulo de matéria orgânica no solo e atividade microbiana, resultando em uma maior disponibilidade de nutrientes para as plantas. Estratégias adequadas de manejo, como o tipo de pastejo e a aplicação de fertilizantes, desempenham um papel fundamental na otimização da absorção de nutrientes e na minimização de perdas nos SIPA. Assim, esse tipo de produção integrada, se apresenta como uma estratégia sustentável e eficiente para melhorar a qualidade do solo, a fim de promover a ciclagem de nutrientes e aumentar a produtividade agrícola, se mostrando essencial para conservar os recursos naturais, melhorar a saúde do solo e alcançar uma agricultura mais sustentável. No entanto, é necessário continuar investindo em pesquisas adicionais e desenvolver abordagens de manejo personalizadas para maximizar o potencial dos SIPA e implementar práticas agrícolas mais eficientes, visando a sustentabilidade ambiental e a segurança alimentar.

Palavras-chave: Sistemas Integrados de Produção Agropecuária, qualidade do solo, ciclagem de nutrientes, produtividade das culturas, agricultura sustentável.

ABSTRACT

The Integrated Crop-Livestock Systems (ICLS) have awakened a growing interest as a promising approach to diversify agricultural activities and recover only degraded areas. This thesis has the objective of investigating the effects of the ICLS on the quality of the soil, on the cycling of nutrients and on the productivity of crops, in order to promote a more sustainable agriculture. Our results will reveal consistent improvements in various solo properties, such as amount of microbial biomass carbon (MCB), microbial biomass nitrogen (NMB), solo respiration, enzyme activity, pH, total organic carbon (TOC), and nutrient availability. An important highlight was the positive impact of animal grazing on ICLS, which especially influenced the biological properties of solo, indicating better nutrient cycling and better structure of solo. Furthermore, the interaction between animals, plants and the microbial biomass has only shown us ICLS to be beneficial for plant nutrition. The presence of animals in the ICLS areas promotes a constant cycling of nutrients, accumulation of organic matter not only and microbial activity, resulting in a greater availability of nutrients for the plants. Appropriate management strategies, such as the type of pasture and the application of fertilizers, play a fundamental role in optimizing nutrient uptake and minimizing losses in ICLS. Likewise, this type of integrated production is presented as a sustainable and efficient strategy to improve the quality of the soil, in order to promote nutrient cycling and increase agricultural productivity, proving to be essential to conserve natural resources, improve the health of the only to reach a more sustainable agriculture. However, it is necessary to continue investing in additional research and develop personalized management approaches to maximize the potential of ICLS and implement more efficient agricultural practices, aiming at environmental sustainability and food safety.

Keywords: Integrated Agricultural Production Systems, soil quality, nutrient cycling, crop productivity, sustainable agriculture.

LIST OF TABLES

LIST OF FIGURES

CHAPTER 2

CHAPTER 3

LIST OF ABBREVIATIONS AND ACRONYMS

MBN	microbial biomass nitrogen
MBC	microbial biomass carbon
P	Potassium
K	Potassium
Mg	Magnesium
Al	Aliminium
H+Al	Potential acidity
TOC	Total organic carbon
CEC	Cation exchange capacity
$C-CO_2$	Basal respiration
qCO^2	metabolic quotient
C	Carbon
MCS	Monocrop culture system
CLS	Crop-livestock system
CLFS	Crop-livestock-forestry system
ICLS	Integrated crop-livestock system
NITA	Núcleo de inovação tecnológica em agropecuária (Portuguese) or Agriculture technological Imovation center (English)
NNI	Nitrogen Nutrition Index
CCA	Canonical correlation analysis
NH_4^+	Ammonium

SUMARY

GENERAL INTRODUCTION

Integrated Crop-Livestock Systems (ICLS) are based on the intensive exploitation of agricultural areas, which, supported by conservationist principles, differ from other systems due to the sustainability promoted over time. The benefits of adopting integrated systems range from greater diversification of activities that can take place in the same area, to benefits related to improvements in soil quality.

Some studies have already reported improvements in plant and soil nutrition (Moraes et al., 2019; Sarto et al., 2020; Valani et al., 2020; Franzluebbers, 2018; Franzluebbers, 2020), which occur mainly due to the greater deposition of plant residues and animal excreta, which can modify the flow of nutrients between soil, plant and atmosphere (Anghinoni et al., 2013). Although the presence of animals is the main agent of change, studies on their impact on the soil have only become more in-depth in recent years, generally associated with economic issues or used to determine some effect of animals on the soil.

Among the effects of the presence of animals in agricultural areas are higher crop yields, a catalytic effect on the flow of nutrients, improvements in water flow, greater microbial diversity, a greater stock of C and N, an increase in soil organic matter, and consequently greater efficiency in the use of fertilizers and natural resources (Sarto et al., 2020).

In this context, the soil seems to be the main compartment of the processes that occur in the soil-plant-atmosphere system, because while plants produce energy, animals introduce new nutrient pathways and promote spatial changes in soil properties (Sarto et al., 2020). Thus, understanding the effect of the presence of animals in these areas becomes extremely necessary in order to understand the changes that occur in the soil as processes and nutrient flows are affected.

1.1 HYPOTHESES AND OBJECTIVES

The hypothesis of this thesis is that early nitrogen fertilization of the pasture in conjunction with grazing enhances the availability of nitrogen, reducing competition between the soil's microbial biomass and the maize crop for the nutrient.

The general objective of this thesis was to evaluate the impact of the presence of grazing animals on the biological attributes of the soil, as well as to investigate the resulting effect on the Nitrogen Nutrition Index of the maize crop.

1.2 SPECIFIC OBJECTIVES

- Evaluate soil microbial biomass carbon and nitrogen (CMB and NMB, respectively) in ICLS and monoculture areas;
- Evaluate the Nitrogen Nutrition Index (NNI) of the maize crop in ICLS and monoculture areas;
- Compare the NNI, CMB and NMB in ICLS and monoculture areas.

OVERVIEW

All the studies in this thesis were carried out in experimental areas at UFPR. A large part of the studies were carried out at the Núcleo de Inovação Tecnológica em Agropecuária - NITA, which is part of a research group at the Federal University of Paraná, while the other part was developed at Fazenda Capão Redondo.

These areas of study play a fundamental role in disseminating knowledge about Integrated Crop-Livestock Systems. In addition, NITA is one of the main research groups of the ICLS Alliance and plays a key role in the project "Crop-Livestock Integration: Systems for promoting sustainability in an area of environmental protection" and good agricultural practices (Dominschek et al., 2018).

Chapter 1 presents a meta-analysis on studies conducted with ICLS comparing areas with and without grazing animals, and their effects on the physical, chemical and biological attributes of the soil. To do this, data was acquired from a search in the library of peer-reviewed articles on the "Web of science", "Scopus", "Science direct" and "Google Scholar" platforms, using the keywords: "ICLS" and "Soil biomass activity".

In Chapter 2, we conducted a field study on the influence of eucalyptus (E. benthamii) on the CMB, NMB and NNI of the corn crop. The data was collected during the 2019/2020 harvest. This chapter was published in the International Journal of Plant Production (Ruthes et al., 2023).

Finally, the 3rd chapter presents an analysis of the soil and plants in ICLS and monoculture areas. To do this, we evaluated the CMB, NNI - of winter grasses and the corn crop at two different sites, NITA and Fazenda Capão Redondo. The data was collected in the winter of 2020 and in the 2021/2022 harvest.

Funding

This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Funding Code 001.

CHAPTER 1

META-ANALYSIS SHOW BENEFITS OF CATTLE GRAZING ON THE SOIL QUALITY BIOLOGICAL INDICATORS UNDER INTEGRATED CROP-LIVESTOCK SYSTEMS IN THE TROPICS

Meta-analysis show benefits of cattle grazing on the soil quality biological indicators under integrated crop-livestock systems in the tropics

ABSTRACT

Integrated Crop-Livestock Systems (ICLS) are excellent alternative for diversifying activities and restoring degraded soils. Our meta-analysis encompassed a broad range of scientific studies that investigated the effects of ICLS on various soil attributes, such as soil microbial biomass carbon (MB-C), microbial biomass nitrogen (MB-N), soil respiration, metabolic quotient, soil enzymes, pH, total organic carbon (TOC), soil nutrient (available P, Ca+2, K+), and soil density in four different edaphoclimatic conditions (tropical climate loamy or sandy soil, subtropical climate loamy soil, and cold continental climate sandy soil). The meta-analysis results consistently indicated improvements in most of the studied attributes when ICLS was adopted. Animal grazing in the ICLS proved to have a particularly impactful effect on the attributes of MB-N, and the soil enzyme activity. These findings suggest that the presence of animals in ICLS areas can result in better nutrient cycling and soil structure improvement. Additionally, it led to a significant enhancement of soil chemical attributes, such as TOC and nutrient availability. Our results highlight that ICLS can be a sustainable strategy to improve soil quality, positively influencing both microbiological and chemical and physical aspects, which underscore the importance of adopting strategies that integrate different agricultural and livestock activities, aiming at conserving natural resources and increasing productivity in a sustainable manner.

The meta-analysis

Integrated Crop-Livestock Systems (ICLS) promote diversification of farm activities, leading to sTable income and potential improvements of soil quality and plant nutrition (Moraes et al., 2019; Sarto et al., 2020; Valani et al., 2020; Franzluebbers, 2018; 2020; Ruthes et al., 2023). However, ICLS have more elements and are more complex systems than monoculture or extensive cattle ranching, and the interactions between different elements such as crop growth and livestock grazing are not fully understood.

We performed a meta-analysis to confirm the effects of livestock grazing on soil physical, chemical, and biological indicators under ICLS in the tropics and subtropics to verify the effects of ICLS on soil quality. The data were acquired through a search of peer-reviewed articles in the databases "Web of Science," "Scopus," "Science Direct," and "Google Scholar," using the keywords "integrated crop-livestock systems” and "soil microbial biomass", and “soil microbial activity”. From the compiled library (Supplementary Material S1), we extracted the mean values, standard deviations, and the number of replicates for the measurements of soil microbial biomass and activities, as well as the other soil biological, chemical, and physical indicators presented in each study. Studies with fewer than three replicates were excluded from the meta-analysis. When the standard deviation was not presented in the article, we calculated it from the coefficient of variation or standard error using stablished statistical methods. If neither of these indicators was presented, we estimated them using the available means. In the meta-analysis, systems without grazing were considered "control," and systems with grazing livestock were considered the "treatment." The effect size was calculated using logarithmic response ratios (lr) and the total variability in response ratios from the control and experimental groups, using the equations proposed by Hedges et al. (1999) and Gurevitch & Hedges (2001). Statistical analyses were performed using the metafor package in R software version 4.0.1 (Viechtbauer, 2010), with the script adapted from Kaschuk et al. (2022).

Our meta-analysis confirmed that the adoption of ICLS increases the C and the N of soil microbial biomasses (BMC: *lr*=0.1322, and BMN: *lr*=0.2625 respectively) (Table 1). Measurements of BMC and BMN give information about the living soil mass of organisms smaller than 103 μm^3 (Jenkinson and Ladd, 1981, Kaschuk et al., 2010), which quickly respond to managemt changes in agricultural systems (Kavamura et al., 2019; Anzalone et al., 2020; Lazeris et al., 2021; Ferreira et al., 2021; Ruthes et al. 2023). Although the CMB represents only 1-3% of soil organic C, it is the most active fraction of soil organic matter and contribute to various processes such as in the nutrient cycling and in the soil organic matter (SOM) formation (George et al., 2013). Animal grazing stimulates the roots of forage plants to grow as cutting of the aboveground plant parts generates new meristems with greater root density and biomass (George et al., 2013; Ambus et al., 2018; Adetunji et al., 2020; Turmel et al., 2015). Root growth increases the input of organic C into the soil and thereby affects soil physical, chemical, and microbiological indicators (Anghinoni et al., 2013; Ambus et al., 2018; Paes et al. 2018; Gamboa et al., 2020; Martins et al., 2020). Furthermore, animal feces and urine increase available N contributing to plant growth and increased amounts of NMB (Hendrickson & Sanderson, 2017; Ruthes et al., 2023).

Table 1 - Logarithmic response ratio of the microbiological, chemical, physical and enzyme attributes of the soil submitted to ICLS.

Soil attribute #	**Experimental condition**	*n*	*lr*	*95% C.I*	*p*
CMB		129	0.1322	0.0646; 0.1999	0.0001
NMB		46	0.2625	0.1686;0.3564	<0.0001
C-CO_2		42	0.0662	0.0067;0.1256	0.0292
qCO_2		62	-0.0159	-0.1052;0.0734	0.7273
qMic		18	0.1267	0.0281;0.2253	0.0118
Urease		34	0.1916	0.1092;0.2740	<0.0001
Arylsulfatase		22	0.1964	0.0680;0.3248	0.0027
Beta-glucosidade		77	0.0992	0.0422;0.1529	0.0003
Acid phosphatase		51	-0.0034	-0.0421;0.0353	0.8636
Alcaline phosphatase		35	0.3808	0.1497;0.6118	0.0012
Arylamidase		11	0.8843	0.5351;1.2334	<0.0001
Beta-glucosaminidase		12	0.5554	0.2564;0.8545	0.0003
pH		43	-0.0133	-0.0324;0.0057	0.1705
COT	ICLS	88	0.0845	0.0504;0.1185	<0.0001
P		37	0.0066	-0.1725;0.1857	0.9424
Ca		16	0.2784	-0.1344;0.4225	0.0002
CEC		8	0.0812	-0.0354;0.0719	0.5054
K		35	0.0626	-0.448;0.1701	0.2532
Mg		16	0.2812	0.2019;0.3605	<0.0001
SB		8	0.2313	0.1096;0.3530	0.0002
SOM		62	0.1785	0.1282;0.2288	<0.0001
TN		58	0.0054	-0.0139;0.0247	0.5840
H+Al		4	-0.2521	-0.3414;-0.1628	<0.0001
Al		7	0.9522	0.1803;1.7241	0.00156
Macroporosity		1	0.000	-0.1782;0.1782	1.0000
Microporosity		3	0.0075	-0.0073;0.0223	0.3182
Soil density		31	0.0205	-0.0061;0.0471	0.1306

n is the number of observations; *lr* is the logarithmic response ratio obtained by dividing the treatment value (ICLS) by the control (no grazing); 95% C.I are the lower and upper confidence intervals at p<0.95;

Interpretation: Negative values of *lr* indicated that the adoption of the ICLS system decreases the values of the variables and positive values of *lr* indicated that the adoption of the ICLS system promoted increases in the values of the variables.

When the *lr* value is negative and both confidence intervals are negative, the effects are significantly negative. When the *lr* value is positive and both confidence intervals are positive, the effects are significantly positive.

Soil CO_2 emissions, metabolic quotient (qCO_2), and microbial quotient (qMic) are microbiological indicators that outline metabolic adjustament to environmental changes (Anderson and Domsch, 1993). This meta-analysis revealed that cattle grazing increases soil CO_2 emissions (*lr*=0.0.0662) and qMic (*lr*=0.1237) (Table 1). The ICLS systems accumulate more organic residues through feces and urine while cattle grazing stimulates root growth and C exudation (George et al., 2013), which stimulates microbial respiration (CO_2 emissions) and growth (qMic) (Hou et al., 2019). The most interesting finding, however, is the fact that qCO_2 is preserved, which suggests that cattle grazing is not causing soil microbial biomass to divert energy from growth and production to maintenance (Anderson and Domsch, 1993; Kaschuk et al. 2010, Anzalone et al., 2020). Consequently, more C can be allocated into MBC, total organic C, and SOM (Table 1).

In fact, the formation of SOM is a complex process that depends on various intrinsic and extrinsic factors of the soil ecosystem. ICLS can stimulate the activity of saprophytic microorganisms (Shekaran et al., 2021), activating various soil enzymes, such as urease, arylsulfatase, glucosidase, alkaline phosphatase, arylamidase, and glucosaminidase ICLS (Table 1). Higher enzimatic activity suggests that ICLS can promote the decomposition of SOM, leading to the release of carbohydrates, amino acids, orthophosphate ions, and sulfates (Acosta-Martínez et al., 2008; Shekaran et al., 2021), which contribute to the formation of clay-humus complexes and improvements in soil structure (Acosta-Martinez & Tabatabai, 2001; Zhang et al., 2015; Shekaran et al., 2021). Similar to other agricultural systems that prioritize production and incorporation of high-quality residues in the systems (Bayer et al., 2006; Veloso et al., 2018; Cherubin et al., 2020), the meta-analysis confirmed that the adoption of ICLS had a positive impact on SOM content (*lr*= 0.1785; $p<0.0001$).

Soil acidification is a major problem in agriculture worldwide and can result from the leaching of N fertilizers, which releases H^+ ions, increases the availability of Al3+ ions, and decreases nutrient cations in the soil solution (Alves et al., 2019; Martins et al., 2020). The meta-analysis showed that adopting ICLS did not change soil pH, decreased potential acidity (H+Al), and increased Al_3^+ availability (Table 1).

Previous studies (Martins et al., 2014; 2016) have demonstrated that moderate cattle grazing can decrease soil acidification and stabilize pH over time. The meta-analysis also showed that ICLS increased the availability of Ca, Mg, and the sum of bases (Table 1). This could be attributed to improved nutrient cycling due to excreta deposition, increased root exudation and growth, and increased soil microbial activity (Carvalho et al., 2010; Moraes et al., 2014; Martins et al., 2020), making plants and microorganisms more efficient at taking up N, preventing leaching and soil acidification (Menzies, 2003; Rossielo and Jacob-Neto, 2006; Martins et al., 2020). Moreover, it is interesting to highlight that this study did not enconter differences in P, K, N, and CTC between ICLS and monocropping systems (Table 1). This fact reinforce the theory that ICLS contributes to preservation of soil fertility, despite slightly different nutrient dynamics (Costa et al., 2014, Anghinoni et al., 2013).

On average, responses calculated with the data compiled in this study showed that ICLS does not change soil density (Table 1). However, probably due to increased microbial activity and root growth, ICLS stimulates the creation of continuous pores (Rabot et al., 2018; Schlüter et al., 2020) and improves soil physical indicators such as porosity and water content (Table 1). Improvements in soil biological, chemical, and physical characteristics should increase grain yield, as it has been often observed (Sarto et al., 2020). Therefore, we conclude that ICLS systematically improves soil quality and clearly contributes to agricultural sustainability whenever it is possible to be implemented.

Aknowlegments

Authors aknowlegde financial support from Graduate Support Program (PROAP/UFPR), from the the National Council for Scientific and Technological Development (CNPq) through the call Universal 420140/2016-6, and B.E.S.R aknowlegdes a PhD scholarship from Coordination for the Improvement of Higher Education Personnel (CAPES).

Reference

Anghinoni, I, Carvalho, P.C.D.F., Valadão, S.E, Costa, G. A. (2013). Abordagem sistêmica do solo em Sistemas Integrados de Produção Agrícola e Pecuária no subtrópico brasileiro. Tópicos em Ciência do Solo, pp. 325-380

Adetunji, A.T., Ncube, B., Mulidzi, R., Lewu, F.B. (2020). Management impact and benefit of cover crops on soil quality: A review. Soil Tillage Reach., 204:104717, DOI: 10.1016/j.still.2020.104717

Anzalone, R., Vezzani, F.M., Kaschuk, G., Hungria, M., Vargas, L., Nogueira, M.A. (2020). Establishing reference values for soil microbial biomass-C in agroecosystems in the Atlantic Forest Biome in Southern Brazil. Ecological Indicators, 117:106586. https://doi: 101016/j.ecolind.2020.106586

Adetunji, A. T. Ncube, B., Mulidzi R., Lewu, F.B.(2020). Management impact and benefit of cover crops on soil quality: a review. Soil & Tillage Research, 204:104717. Doi: https://doi.org/10.1016/j.still.2020.104717

Alves, L.A, Denardin, L.G.O., Martins, A.P., Anghinoni, I., Carvalho, P.C.F., Tiecher, T. Soil acidification and P, K, Ca, and Mg budget as affected by sheep grazing and crop rotation in a long-term integrated crop–livestock system in southern Brazil (2019). Geoderma. 351:197-208. https://doi.org/10.1016/j.geoderma.2019.04.036

Ambus, J. V. et al. Changes in composition and functional soil properties in long-term no-till integrated crop-livestock system (2018). Geoderma, 330: 232-243. doi: 10.1016/j.geoderma.2018.06.005.

Acosta-Martinez, V., Tabatabai, M., (2001). Tillage and residue management effects on arylamidase activity in soils. Biology and Fertility of Soils. Vol. 34 (1):21-24.

Bayer, C., Lovato, T., Diekow, J., Zanatta, J.A., Mielniczuk, J. (2006). Um método para estimar coeficientes de dinâmica da matéria orgânica do solo com base em experimentos de longo prazo Soil Tillage Res., 91:217-236

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

Franzluebbers, A. (2020). Soil-test biological activity with the flush of CO2: Validation of nitrogen prediction for maize production. Agronomy Journal, 112:2188-2204. https://doi: 10.1002/agj2.20094

Franzluebbers, A. (2018). Soil-test biological activity with the flush of CO2: III Maize yield responses to applied nitrogen. Soil Science Society of America Journal, 82:708-721. Htts://doi:10.2136/sssaj2018.01.0029

Ferreira, G.W.D., Rau, B.M., Aubrey, D.P. (2021). Temporal nitrogen dynamics in intensively managed loblolly pine early stand development. Forest Ecology Management, 48:118890. https://doi: 10.1016/j.foreco.2020.118890

Gurevitch J, Hedges LV (2001) Meta-análise: combinando os resultados de experimentos independentes. In: Scheneir SM, Gurevitch J (eds) Desenhos e análise de experimentos ecológicos, 2ª edn. Oxford University Press, Oxford, pp 347–369

Gamboa, C.H., Vezzani, F.M., Kaschuk, G., Favaretto, N., Cobos, J.Y.G., Costa, G.A. (2020). Soil-Root Dynamics in Maize-Beans-Eggplant Intercropping System under Organic Management in a Subtropical Region. Journal Soil Science and Plant Nutrition, 20:1480–1490. https://doi:10.1007/s42729-020-00227-9

Hendrickson J., Sanderson M., Chapter 7 - Perennial-Based Agricultural Systems and Livestock Impact on Soil and Ecological Services, Editor(s): Mahdi M. Al-Kaisi, Birl Lowery, Soil Health and Intensification of Agroecosytems, Academic Press, 2017, Pages 151-171, ISBN 9780128053171, https://doi.org/10.1016/B978-0-12-805317-1.00007-5.

Hou, P.F., Chien, C.H., Chiang-Hsieh, Y.F., Tseng, K.C., Chow, C.N., Huang, H.J., Chang, W.C., 2018. Paddy-upland rotation for sustainable agriculture with regards to diverse soil microbial community. Sci. Rep. 8, 1–9.

Hedges, L.V.; Gurevitch, J.; Curtis, P.S. (1999). The meta-analysis of response ratios in experimental ecology. Ecol. 80:1150–1156. Doi: https://doi.org/10.1890/0012-9658(1999)080[1150:TMAORR]2.0.CO;2

Kaschuk, G., Galli-Terasawa, L.V., Albernas, K.K., Ripka, L.P., Pansolin, I.C., Matos, C.L., Pauletti, V., Kava, V. (2022). Inoculation of plant growth promoting Bacillus spp. in N-fertilized maize crop in soils with high organic matter content in South Brazil. Journal Agriculture Science, 14: 43-52 https://doi.org/10.5539/jas.v14n7p43

Kaschuk, G., Alberton, O., Hungria, M. (2010). Three decades of soil microbial biomass studies in Brazilian ecosystems: Lessons learned about soil quality and indicators for improving sustainability. Soil Biol. Biochem. 42:1-13. https://doi.org/10.1016/j.soilbio.2009.08.020

Kavamura V.N., Robinson, R.J., Hayat, R., Clark, I.M., Hughes, D., Rossmann, M., Hirsch, P., Mendes, R., Mauhilne,T. (2019). Land management and microbial seed load effect on rhizosphere and endosphere bacterial community assembly in wheat. Front Microbiol, 10 (2019), p. 2625, 10.3389/fmicb.2019.02625

Lazeris, T.S., Souza, J.P., Vezzani, F.M., Lima, C.M., Kaschuk, G. (2021). Carbon and phosphorus biogeochemical cycles in native forest and horticultural areas in the Metropolitan Region of Curitiba, Brazil. Colloquium Agrariae, 17:1-^{1}1. https://doi:10.5747/ca.2021

Moraes, A., Carvalho, P.C.F., Anghinoni, I., Lustosa, S.B.C., Costa, S.E.V.G.A., Kunrath, T.R. (2014). Integrated crop–livestock systems in the Brazilian subtropics. European Journal of Agronomy, 57:4–9. https://doi:10.1016/j.eja.2013.10.004

Moraes, A., Carvalho, P.C.F., Crusciol, C.A.C., Lang, C.R., Pariz, C.M., Deiss, L., Sulc, M.R. (2019). Integrated Crop-Livestock Systems as a Solution Facing the Destruction of Pampa and Cerrado Biomes in South America by Intensive Monoculture Systems. In: Agroecosystem Diversity: Reconciling Contemporary Agriculture and Environmental Quality. Elsevier: Amsterdam, The Netherlands. p.257–273, doi: https://doi: 10.1016/B978-0-^{1}2-811050-8.00016-9

Martins, A.P., Denardin, L.G.O.,Tiecher, T., Borin, J.B.M., Schaidhauer, W.S., Anghinoni, I., Carvalho, P. C. F., Kumar, S. (2020). Nine-year impact of grazing management on soil acidity and aluminum speciation and fractionation in a long-term no-till integrated crop-livestock system in the subtropics. Geoderma, v. 359:113986, doi: https://doi.org/10.1016/j.geoderma.2019.113986

Martins, A. P., Costa, S. E. V. G. A., Anghinoni, I., Kunrath, T. R.,Balerini, F., Cecagno, D. , Carvalho, P. C. F. (2014). Soil acidification and basic cation use efficiency in an integrated no-till crop-livestock system under different grazing intensities. Agriculture Ecosystems & Environment , v. 195, p. 18-28. Doi: https://doi.org/10.1016/j.geoderma.2019.113986

Martins, A.P., Cecagno, A.P. D., Borin, J.B.M., Arnuti, F., Lochmann, S.H., Anghinoni, I., Bissani, C.A., Bayer, C., Carvalho, P.C.F. (2016). Long-, medium- and short-term dynamics of soil acidity in an integrated crop–livestock system under different grazing intensities. Nutr. Cycl. Agroecosys., 104: 67-77. Doi: https://10.1007/s10705-015-9759-5

R Core Team. R: A language and environment for statistical computing. (2022). Vienna, Austria, R Foundation for Statistical Computing. Versão 4.0.2.

Sarto, M.V.M., Borges, W.L.B., Sartom J.R.W., Rice, C.W., Rosolem, C.A. (2020). Root and shoot interactions in a tropical integrated crop–livestock–forest system. Agricultural Systems, 181:102796. https: doi://101016/j.agsy2020.102796

D. S. Jenkinson, J. N. Ladd. (1981). Microbial biomass in soil: measurement and turnover. 1st Edition. ISBN: 9781003064763

Paes, L.S.D.O.P., Lima, M.R., Kaschuk, G., Vezzani, F.M. (2018). Root development as an indicator of soil sustainability of cropping systems in the Atlantic Forest Biome. Cultivos tropicales, 39: 7-14

Rossielo, R.O.P., Jacob Neto, J. (2006). Toxidez de alumínio em plantas: novos enfoques para um velho problema M.S. Fernandes (Ed.), Nutrição mineral de plantas, Sociedade Brasileira de Ciência do Solo, Viçosa. pp. 375-418

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

Schlüter, S., Sammartino, S., Koestel, J. (2020). Exploring the relationship between soil structure and soil functions via pore-scale imaging, Geoderma. p. 370, DOI: 10.1016/j.geoderma.2020.114370

Turmel, M.S., Speratti, A., Baudron, F., Verhulst, N., Govaerts, B. (2015). Crop residue management and soil health: a systems analysis. Agric. Syst., 134:6-16, DOI: 10.1016/j.agsy.2014.05.009

Valani, G.P., Martíni, A.F., Silva, L.F.S., Bovi, R.C., Cooper. M. (2020). Soil quality assessments in integrated crop–livestock–forest systems: A review. Soil Use and Management, 37:22-36, doi: https://doi:10.1111/sum12667

Viechtbauer W (2010) Realização de meta-análises em R com o pacote metafor. J Software Estatista 36:1–48. https://doi.org/10.18637/jss.v036.i03

Veloso, M.G. Angers D.A., Tiecher, T., Giacomini, S. Dieckow, J., Bayer, C. (2018). Alto armazenamento de carbono em solo subtropical previamente degradado sob plantio direto com culturas de cobertura de leguminosas. Agric. Ecosyst. Environ., 268:15-23, DOI: 10.1016/j.agee.2018.08.024

Zhang T, Zhang YJ, Xu MJ et al. (2015b) Light-intensity grazing improves aICLSine mea dow productivity and adaption to climate change on the Tibetan Plateau. Scientific Reports, 5, 15949

CHAPTER 2

SOIL MICROBIAL BIOMASS, N NUTRITION INDEX, AND YIELD OF MAIZE CULTIVATED UNDER EUCALYPTUS SHADE IN INTEGRATED CROP-LIVESTOCK-FORESTRY SYSTEMS[1]

This chapter is presented according to the International Journal of Plant Production guidelines. Manuscript published online: April, 13 2023. https://doi.org/10.1007/s42106-023-00242-7. Authors: Barbara Elis Santos Ruthes, Glaciela Kaschuk, Anibal de Moraes, Claudete Reisdorfer Lang, Camila Crestani and Leandro Bittencourt de Oliveira

Soil microbial biomass, n nutrition index, and yield of maize cultivated under eucalyptus shade in integrated crop-livestock-forestry systems

ABSTRACT

Integrated crop-livestock-forestry systems are promising alternatives to the landscape impoverishment caused by monoculture systems. However, nutrient management in integrated systems still needs to be resolved better. As the soil microbial biomass (MB) is a soil nutrient reservoir that can contribute to crop nutrition, we aimed to understand the relationships between C and N of soil MB (CMB and NMB, respectively), the N nutrition index (NNI), and crop yield in monoculture and integrated crop-forestry, crop-livestock, and crop-livestock-forestry systems to propose management strategies for N fertilization in integrated systems. The systems were established in an experiment at the Center for Technological NNIovation in Agriculture (Portuguese acronym: NITA) in 2012 under a randomized block design with three repetitions, with maize as the crop, Angus beef cattle as livestock, and eucalyptus as the forestry components. Measurements were made in 2019. The systems resulted in satisfactory values of CMB and NMB; nonetheless, the crop-forestry system had a higher CMB (338.7 mg kg^{-1}) than the monoculture system (272.7 mg kg^{-1}). The NMB was increased by N fertilization by 22% in all systems, and it was higher in the crop-livestock and crop-livestock-forestry systems, probably due to the deposition of N-rich livestock urine. The N-fertilization also increased crop yield and NNI in the monoculture crop system but not in the crop-forestry system, probably because trees formed a shady ecosystem that limited plant growth. Thus, the results indicate that Tables for N fertilization must consider the specific edaphoclimatic conditions of integrated crop-livestock-forestry to avoid excessive N fertilization rates.

Keywords: soil quality, sustainable agricultural systems, subtropical climate, eucalyptus.

1. Introduction

Integrated agricultural systems are promising alternatives to landscape impoverishment caused by monoculture systems (MCS) (Lemaire et al., 2014;

Moraes et al., 2019; Peterson et al., 2020; Rufino et al., 2021; Sekaran et al., 2021). Integrated agricultural systems organize annual crop production with different possible arrangements, such as intercropping, succession or rotation, and consortium with perennial crops or trees, pastures, and grazing animals in the same area (Moraes et al., 2019; Valani et al., 2020). They usually have three components: crop, livestock, and forestry; each contributes complementarily to agricultural sustainability. Following that, integrated agricultural systems generate additional and more sTable income than MCS (Lemaire et al., 2014; Pacín and Oesterheld, 2014; Moraes et al., 2019). Therefore, integrated agricultural systems (crop-livestock system - CLS; crop-forestry system - CFS; and crop-livestock-forestry system - CLFS) increase agrobiodiversity and improve the soil environment conditions for plant growth and soil biological activity (Lemaire et al., 2014; Moraes et al., 2019; Bieluczyk et al., 2020; Sarto et al., 2020; Valani et al., 2020).

However, despite the known benefits (Moraes et al., 2014; 2019), integrated CLS, CFS, and CLFS do not compose the mainstream of agricultural production (van Keulen and Schiere, 2004; Lemaire et al., 2014; Bieluczyk et al., 2020; Souza-Jr. et al., 2020). For example, in Brazil, only 11% of livestock production comes from CLS and CLFS (Bieluczyk et al., 2020; Santos et al., 2020). The low adoption of integrated agricultural systems is probably related to the fact that farmers do not encounter adequate rural credit policy (Carrer et al., 2020) and do not reach reliable information on the management strategies of integrated systems (Perosa et al., 2021), and/or the strategies for intensifying integrated agricultural systems are poorly developed (Rufino et al., 2021), for which more research should be dedicated. In other words, the limiting factors of these systems (including changes in edaphoclimatic conditions) need to be better understood (Valani et al., 2020; Rufino et al., 2021). Most studies have been designed to explain crop responses under MCS, and there is little information in the literature regarding the effects of livestock and forestry on crop responses to soil fertilization under integrated agricultural systems.

Analyzing the effects of integrated CLFS on crop responses to soil fertilization is a challenging task. For example, introducing trees in the CFS and CLFS promotes soil microbial diversity, which can directly affect crop growth (Moraes et al., 2019; Sarto et al., 2020; Valani et al., 2020; Franzluebbers, 2018; Franzluebbers, 2020). However, the trees in the CLFS and CFS may change light interception distribution in the area (Pezzopane et al., 2020; Glatzle et al., 2021; Kruchelski et al., 2021), and thus affect crop photosynthetic capacity and plant N metabolism (Fu, 2017; Liang, 2020; Lu, 2013; Wei, 2018; Geremia et al., 2018). The inclusion of cattle in the integrated CLS promotes the return of nutrients to the system by the deposition of feces and urine, modifying the nutrients' biogeocycles, stimulating soil microbial biomass, and improving soil physical structure and moisture (Sarto et al., 2020; Valani et al., 2020; Deiss et al., 2019; Bernardon et al., 2020; Bieluczyk et al., 2020). Additionally, the presence of livestock would imply the presence of grasses for grazing, which produce a greater volume of roots and a higher exudation of organic compounds than crops, leading to increases in soil microbial biomass and organic matter (Paes et al., 2018; Gamboa et al., 2020; Valani et al., 2020). However, excessive livestock trampling may damage the soil's physical characteristics by compacting soil sublayers (Moraes et al., 2014). Thus, such a paradoxical scenario calls for further studies to recognize the strengths and limitations of integrated agricultural systems.

Soil microbial biomass is a reservoir of soil nutrients and can contribute to plant nutrition through coupled C and N biogeocycling (Kaschuk et al., 2010; Anzalone et al., 2020; Lazeris et al., 2021; Ferreira et al., 2021). Therefore, the C and N of soil microbial biomass (CMB and NMB, respectively) correlate with increasing crop yields, while they respond faster than other soil variables, including total organic carbon, to changes in the soil environmental conditions (Kaschuk et al., 2010; Anzalone et al., 2020; Franzluebbers, 2018; Franzluebbers, 2020; Ferreira et al., 2021).

We chose to evaluate the maize (Zea mays L.) crop for this study. When maize is included in crop rotation systems, it improves soil quality due to the growth of a large volume of roots, improving the soil's physical, chemical, and biological characteristics (Paes et al., 2018; Gamboa et al., 2020). Maize is grown for grains, fodder, and silage on large and small farms. To date, in 2021, maize was harvested in 19 Mha of Brazilian agricultural areas (22% of the total area), following soybeans, which occupy 45% of total area, and before sugarcane (11.5%), wheat (3.2%), beans (3%), and rice (2%) (FAOSTAT, 2023). Maize is a crop that responds significantly to variable edaphoclimatic conditions and N fertilization (Kaschuk et al., 2022), and there is a strong positive correlation between maize responses to N fertilization and soil biological activity (Franzluebbers, 2018; Franzluebbers, 2020; Ferreira et al., 2021).

Along these lines, this study hypothesized that the soil's CMB and NMB promptly increase with the addition of N fertilizer and readily ensure more efficient N recycling in the soil ecosystem. Furthermore, increases in the soil's CMB and NMB should increase maize NNI and grain yield.

The main objective was to understand the relationships between CMB and NMB as indicative of soil health and to relate the soil microbiological indicators to the crop N nutrition index (NNI) in areas of MCS, CFS, CLS, and CLFS to propose more adequate rates of N fertilization in these systems.

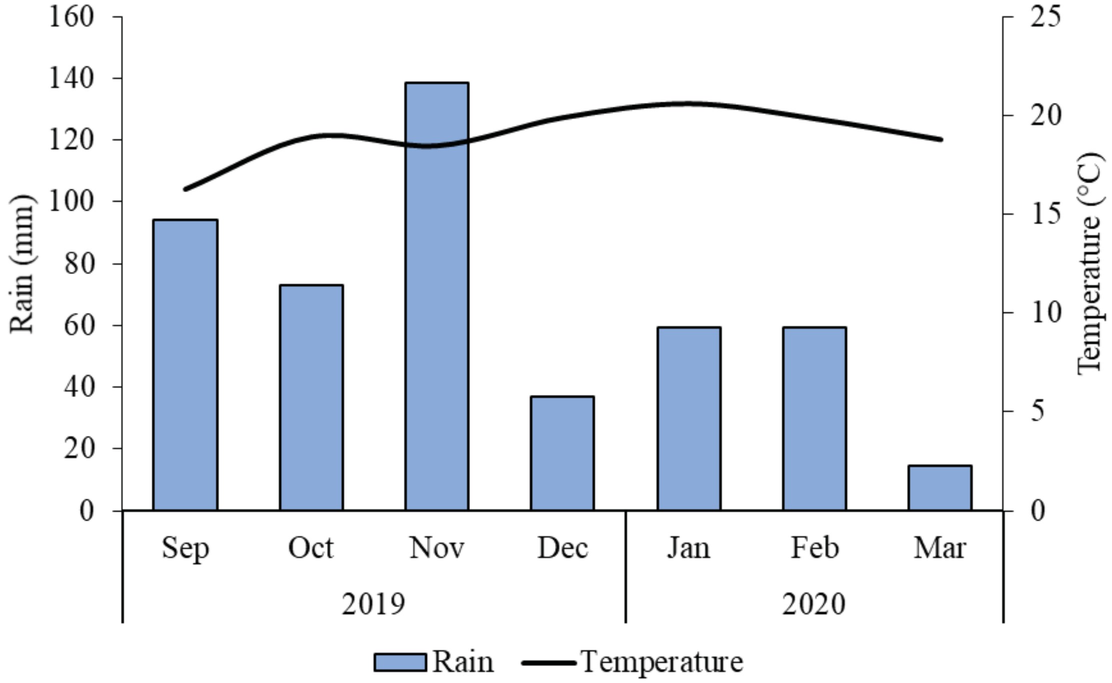
2 Material and methods

2.1 Location

The study was a long-term experiment started in 2012 at the Center for Technological NNIovation in Agriculture (Portuguese acronym: NITA), located at the Experimental Station of Canguiri, belonging to the Federal University of Paraná (UFPR), located in the municipality of Pinhais, Paraná, Brazil. The central coordinates of NITA are 25º 23' 30" south and 49º 07' 30" west, and the average altitude of the location is 920 m. According to Köppen, the climate is of type Cfb, i.e., which means humid temperate with temperate summer without defined dry season and occurrence of frosts in the winter (Alvares et al., 2013).

The experimental period that occurred during the development of the maize crop was characterized by climatic regularity and allowed the good development of crops (Fig. 1). The average maximum and minimum temperatures were 21.4 ºC and 12.3 ºC, respectively, with average daily temperatures of 15 ºC in the winter and 18.4 ºC in the summer.

FIGURE 1 – Meteorological data from automatic weather stations in the experimental area from September 2019 to March 2020



FONTE: The autor (2022).

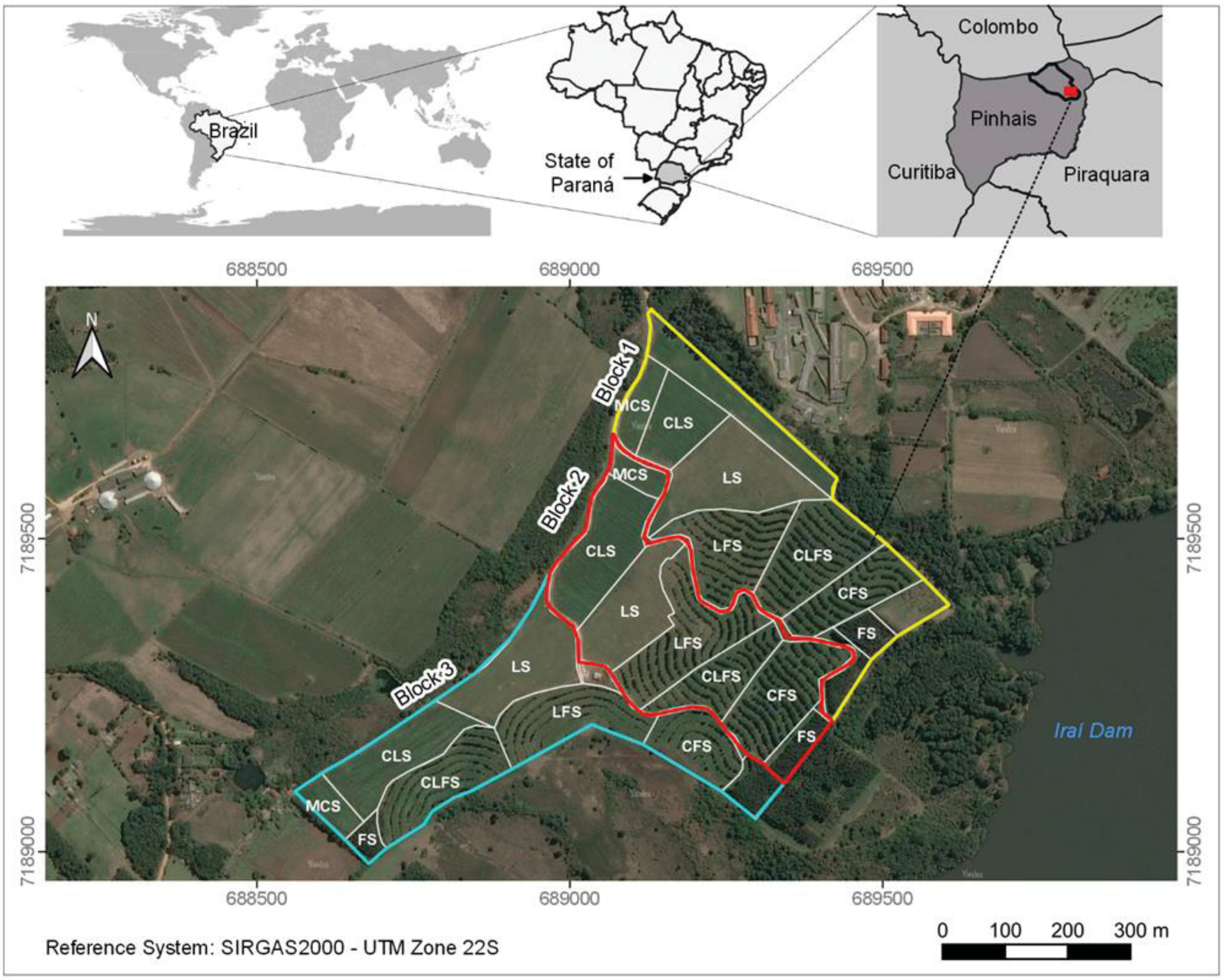
The soil in the current study's experimental area was classified as oxisols (USDA Classification System, 2014), or as typical dystrophic red-yellow latosol in the blocks 1 and 2 (Fig. 2a), and as cambic eutrophic red-yellow latosol in the block 3, according to the Brazilian Classification System (Santos et al., 2018).

2.2 Experimental design for integrated farming systems

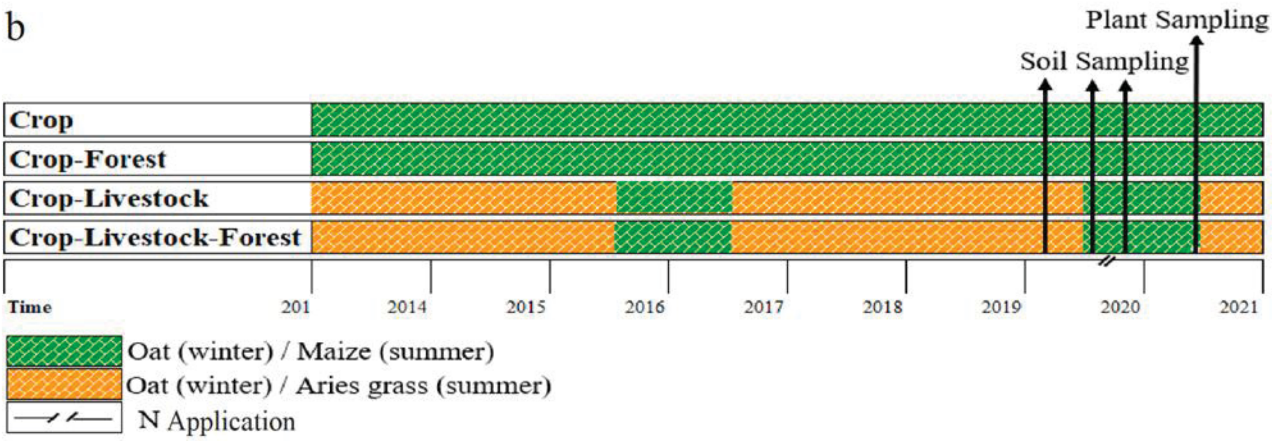
In 2012, the experimental area was tilled with a chisel plow up to 40 cm depth and fertilized with 10 Mg ha^{-1} of N VIRO® applied on the soil surface (Kruchelski et al., 2021). Then in the winter of the same year, black oat (Avena strigosa) was sown with 100 kg P2O5 ha−1 in the planting row over. The treatments of NITA were set up under a randomized complete block design with three replications and seven integrated agricultural systems: monoculture crop (MCS), forestry (FS), livestock (LS), crop-forestry (CFS), crop-livestock (CLS), forestry-livestock (FLS), and crop-livestock-forestry (CLFS). Since then, soil fertilization has been performed evenly across the systems, according to soil analyses and the recommendations of the crops and pastures involved, according to Pauletti and Motta (2019). The sketch of the NITA experiment is presented in Fig. 2a. In the present study, we evaluated four specific treatments: MCS, CLS, CFS, and CLFS, which were chosen due to the presence of the crop at the time. The ordination of these integrated agricultural systems from 2012 to 2019 is presented in Fig. 2b. The MCS was defined as the cultivation of maize for grain production during summer and black oat for soil cover during winter, sown without soil turnover. The integrated CFS was established through crop cultivation between single rows of eucalyptus (Eucalyptus benthamii), planted in a 14-m by 2-m space arrangement of with a planting density of 357 trees ha^{-1}. During three years, the integrated CLS was characterized by the cultivation and grazing of black oat in the winter and guinea grass (Megathyrsus maximus) in the summer, followed by the cultivation of black oat without grazing for a winter period, and finally, by replacement of cultivated pasture with crop inclusion for another three years. The integrated CLFS consisted of making CLS in the area between eucalyptus rows with the same CFS specification. The average animal load (beef cattle) was regulated to be 711 kg ha^{-1} in integrated CLS and 557 kg ha^{-1} in integrated CLFS, with continuous grazing and variable livestock numbers, for approximately 270 days.

FIGURE 2a – Experimental area in the Federal University of Paraná, municipality Pinhais, Paraná, Brazil, and the treatments: monoculture crop system (MCS), livestock system (LS), forestry (FS), crop-forestry system (CFS), livestock-forestry system (LFS), and crop-livestock-forestry system (CLFS). 2b Land use history from June 2012 to March 2020

a



b



2.3 Experiment with n fertilization

The N fertilization experiment was performed when the eucalyptus trees were seven years old, the MCS and CFS were cultivated with maize, and the CLS and CLFS were cultivated with guinea grass and grazed by beef cattle (Fig. 2b). The plots of MCS and CFS, which were grown with maize, were divided into two: the first plot (N+) was fertilized with 135 kg N ha^{-1} applied as urea (45% N), applied by hauling with the aid of a mechanical tractor, and the second plot (100 m^2) was left as a non-fertilized control (N-). The N fertilizer was applied on October 7, 2019, when maize had three leaves fully expanded (V3 stage). However, the CLS and CLFS plots grown with guinea grass, received the entire area's 135 kg N ha^{-1} applied as urea (45% N).

2.4 Samplings

Figure 2b shows the approximate date of soil and plant samplings. Table 1 shows the soil chemical attributes of the systems in samples collected on May 3, 2019, just before the start of this study. Samples for the analyses of soil microbiological indicators (CMB and NMB) were taken in the 0–10 cm layer at ten points within each plot before sowing maize (September 10, 2019) and after N fertilization (October 20, 2019); five samples were taken from the plots with N and five samples from the plots without N. Samples were also collected from the areas grazed by the animals during the time they were grazing, after the fertilization of the pasture, and after the animals left the plots (Figure 2b). For these treatments, ten samples were collected per plot. Shoot samples were taken during the same periods, and grain estimation was determined at the end of the crop cycle.

TABLE 1 - Soil chemical attributes (mean ± standard deviation, n = 3) at 0–10 cm depth in the long-term experiment of different integrated agricultural systems of the Center for Technological NNIovation in Agriculture (Portuguese acronym, NITA), in Pinhais, Paraná, Brazil, in May 2019

System#	pH	P	Organic Matter	K^+	Ca^{2+}	Mg^{2+}	H+ Al	SB	CEC	Crop residues
	$CaCl_2$	mg dm^{-3}	g dm^{-3}	--------cmol dm^{-3}---------						t ha^{-1}
Monoculture Crop	5.00	4.18	40.07	0.39	5.47	2.72	4.44	8.58	13.02	3.6
Crop-Forestry	4.99	5.03	37.32	0.40	4.78	3.12	4.41	8.30	12.71	2.8
Crop-Livestock	5.22	3.50	37.32	0.49	4.85	3.18	3.91	8.52	12.43	1.4
Crop-Livestock-Forestry	5.25	3.84	34.56	0.39	4.64	3.31	3.74	8.34	12.08	1.1

Abbreviations: monoculture crop system (MCS); crop-forestry system (CFS); crop-livestock system (CLS); crop-livestock-forestry system (CLFS). There were no statistical differences between the systems. Crop residues are measured on a dry matter basis, the sum of bases (SB), and cation exchange capacity (CEC). Data were obtained according to the methodology proposed by Santos et al. (2018). In comparison with the guidelines of Pauletti and Motta (2018), the contents of soil P are considered low, those of organic matter are medium, and those of K, Ca, and Mg are high.

2.5 Soil analyses

Soil samples were dried in an oven at 50 °C for 72 h until the mass was constant and passed through a sieve with a 4.75 mm mesh opening to remove roots and impurities. Dried soil samples were stored for three to six months before the analyses were performed. Drying was chosen because the storage of wet soil samples increases the rates of soil microbial death more than the storage of dry soil samples (Gonzalez-Quiñones et al., 2011), probably because microbes consume C stocks more quickly under wet conditions. Moreover, there is evidence that microbial measurements in dried soil samples do not differ statistically from wet soil samples (Haney et al., 2004; Schroeder et al., 2021).

The determination of the CMB was achieved with the fumigation-extraction method of Vance et al. (1987), with minor modifications. For that, sub-samples of about 20 g of dried soil were re-moistened to 50% of the field capacity with deionized water and fumigated with chloroform for 48 hours in a fumigation box attached to the vacuum pump, called “fumigated samples.” During the same period, other soil sub-samples (20 g each) were re-moistened and kept in the dark at room temperature, called “non-fumigated.” For the extraction of C from the soil, the samples received 50 mL of K2SO4 0.5M extract solution and were suspended by stirring at 175 rpm in a horizontal orbital shaker for 60 minutes. Then, the suspensions were centrifuged at

2500 rpm for 10 minutes. At the end, the extract was obtained by filtration on rapid filtration paper (porosity of 7.5 microns). The amount of C extracted from the soil was determined by colorimetry in a spectrophotometer using a 0.5-mL aliquot of the extract. The difference in C determined the CMB found in fumigated and non-fumigated samples multiplied by the correction factor KC = 0.41 (Sampaio et al., 1986).

The NMB was determined in the same extract by wet digestion in a digester block at 350 °C with concentrated sulfuric acid (Bremner, 1965) and by colorimetric determination of ammoniacal N by the indophenol blue method (Feije and Anger, 1972).

The chemical analyses were performed on dried soil samples sieved through a 2 mm mesh, according to Santos et al. (2018). The pH was measured in samples resuspended in 0.01 mol L^{-1} CaCl2. Organic C was determined after wet oxidation with 0.0667 mol L^{-1} K2Cr2O7 and titration by 0.05 mol L^{-1} Fe (NH4)2(SO4)2.6H2O, using diphenylamine as an indicator. Organic matter was obtained by multiplying organic C by 1.724. Soil samples were submitted to sulfuric digestion, and then the available P and K were extracted with Mehlich-^{1} extractor solution (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H2SO4) and determined by spectrophotometry. The exchangeable Al+3, Ca+2, Mg+2, and Al+3 were extracted with 1 mol L^{-1} KCl and determined by atomic absorption spectroscopy.

2.6 Determination of the N Nutrition Index (NNI)

Maize was carried out using the hybrid P1680VHY Leptra® super early with transgenic technology against insects as the area is an environmental preservation area and not meant to be treated with chemical pesticides, with and without 135 kg of N ha^{-1} in the form of urea. The NNI determination of maize was done at the V8 stages. Five plants were collected per linear meter in each treatment to determine dry matter when the crop was in the vegetative phase and had already reached 1 Mg DM ha^{-1}. After that, the entire plant was weighed and dried in an oven with forced air circulation at a temperature of 55 ºC until it reached a constant mass. The dry matter of the whole plant was ground in a Willey mill, and the N concentration was determined by the method of Tedesco (1995). For the NNI estimate, we used the equations presented by Gastal et al. (2015), which establish the relationship between the current N content and the critical N content determined according to the equation: NNI = (% Na)/(% Nc), where % Na corresponds to the current N percentage and % Nc to the critical N.

2.7 Total crop yield

The accumulation of previous crop residues was estimated by sampling and weighting the dry mass with an aliquot in 0.25 m^2, as presented in Table 1. The total crop yield estimated was performed by entire harvesting cobs corresponding to 16 linear meters in the center of each plot in the areas with N application and four linear meters in the areas without N application. Then, the cobs were threshed, and the grains were weighed. The moisture was corrected to 13%.

2.8 Statistical analysis

All variables met the ANOVA assumptions of normality (Shapiro-Wilk test, $P > 0.05$) and homogeneity of variance (Bartlett test, $P > 0.05$). Data were subjected to analysis of variance (ANOVA), considering a split-plot design with “system” as the main plot and “N level” as the subplot. When differences between the studied effects were detected, the means were compared by the Tukey test ($P < 0.05$). Canonical correlation analysis (CCA) was also performed to verify the existing associations between the systems (without N: CSN- and CFSN-, and with N: CSN+ and CFSN+) and the evaluated attributes (NNI, CMB, NMB, CMB:NMB, and crop yield). All statistical analyses were performed using the R software (version 4.0.2; R Core Team, 2022).

3 Results

3.1 C and N of the soil microbial biomass in integrated farming systems

Farming systems and N applications had an impact on the CMB and the NMB. Considering all agricultural systems, the highest value of CMB was measured in the CFS, whereas the lowest one was found in the CLS (Table 2). The NMB values in the systems, including livestock (i.e., CLS and CLFS, 23.5 mg C kg^{-1} soil), were 6.5 mg C kg^{-1} soil higher than the values of NMB in the systems without this component (i.e., CS and CFS, 16.9 mg C kg^{-1} soil) (Table 2). The CMB:NMB ratios were lower in systems with livestock and increased after N fertilization (Table 2). Regarding the N application, the N fertilizer did not affect the soil CMB (Table 2) but increased the NMB by 22% compared to the value before the N application (Table 2).

TABLE 2 - Soil carbon of microbial biomass (CMB) and nitrogen of microbial biomass (NMB) in the long-term experiment of different integrated agricultural systems of the Center for Technological NNIovation in Agriculture (Portuguese acronym, NITA), in Pinhais, Paraná, Brazil, before and after application of N during the growing season of 2019/2020.

Indicators*	CMB	NMB	CMB:NMB
	------------ mg kg^{-1} ------------		
System#			
Monoculture Crop	358.2 ab	16.2 b	21.85 ab
Crop-Forestry	437.0 a	17.6 b	25.47 a
Crop-Livestock	316.4 b	25.0 a	12.95 c
Crop-Livestock-Forestry	403.6 ab	21.9 a	19.01 bc
N fertilizer§			
Before	370.84	18.2 b	17.46 b
After	386.74	22.3 a	22.18 a
CV (%)	14.5	10.05	18.49
***p*-value at F-test**			
System	0.0103	0.0000	0.0030
N fertilizer	0.4899	0.0002	0.0070
System x N fertilizer	0.4007	0.4997	0.5873

*Only means with significant differences are presented. Means followed by small letters in the column and capital letters in the line within the same variable are statistically different according to the Tukey test at p<0.05.

*CLS and CLFS data were obtained from the areas growing pasture, and CS and CFS data were obtained from the areas growing maize.

Samplings were made on 10 September 2019 and 20 October 2019, before and after the N application.

§ We applied 135 kg N ha^{-1}, as urea, on 7 October 2019 in all farming systems.

Corresponding abbreviations: monoculture crop system (MCS); crop-forestry system (CFS); crop-livestock system (CLS); crop-livestock-forestry system (CLFS).

3.2 Relationships between CMB, NMB, NNI, and maize yield in the CFS

Only the systems cultivated with maize (MCS and CFS) were included in this part of the study. Soil microbial analyses revealed that the CFS increased CMB by 66 mg C kg^{-1} soil compared to the MCS; however, it did not affect NMB (Table 3). On the other hand, N application to the crop increased soil CMB by 206.9 mg C kg^{-1} soil and NMB by 10 mg N kg^{-1} soil compared to plots that did not receive N (Table 3).

Table 3 - Soil carbon of microbial biomass (CMB), nitrogen of microbial biomass (NMB) and nitrogen nutrition index (NNI) in the long-term experiment of different integrated agricultural systems of the Center for Technological NNIovation in Agriculture (Portuguese acronym, NITA) in Pinhais, Paraná, Brazil, with or without application of N during the growing season 2019–2020#

Indicator*	CMB	NMB	CMB:NMB	NNI
	-------------- mg kg⁻¹ --------------			
System				
Monoculture Crop	272.7 b	12.9	21.4	0.35 b
Crop-Forestry	338.7 a	14.2	24.3	0.84 a
Fertilizer[§]				
N-	202.2 b	8.6 b	23.6	0.41 b
N+	409.1 a	18.6 a	22.1	0.78 a
CV (%)	9.1	8.6	9.8	19.4
p-value at F-test				
System	0.0060	0.0990	0.0715	0.0200
N fertilizer	0.0000	0.0001	0.2864	0.0070
System × N fertilizer	0.2490	0.5623	0.9911	0.0740

Samplings were made when the Crop and Crop-Forestry systems were cultivated with maize. 135 kg N ha-¹, as urea, was applied to half of the plots on 7 October 2019 and paired soil samples (with and without N application) were collected after 14 days on 20 October 2019. Corresponding abbreviations: monoculture crop system: MCS; crop-forestry system: CFS

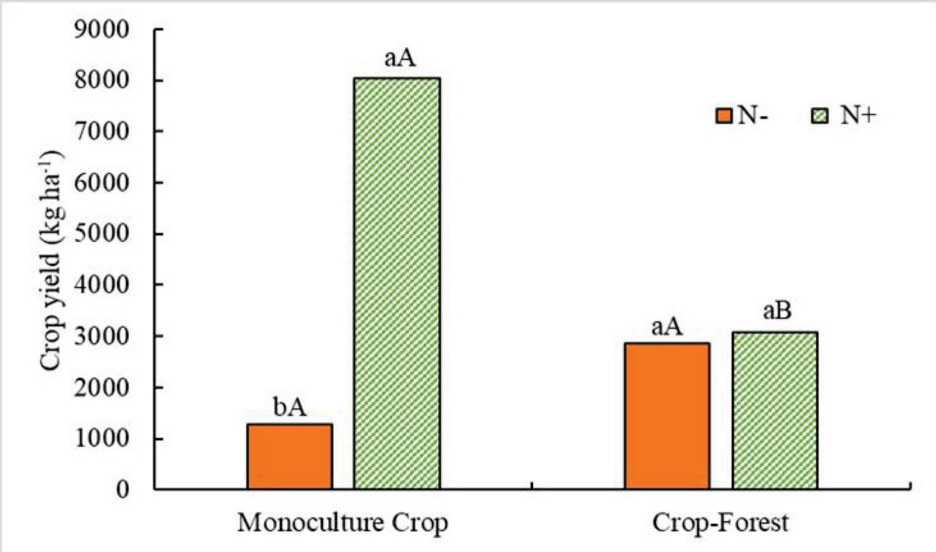
§N+: with 135 kg N ha-¹ fertilization and N-: without N fertilization.

*Means followed by different letters within the same variable in the column highlights the differences between crop systems and in the line between N fertilization treatments and are statistically different, according to the Tukey test at p<0.05

We used the NNI index to determine the N use efficiency of the maize crop in an integrated system. It should be noted that an NNI of 1 means that 100% of crop N demands are met, whereas an NNI lower than 1 means that crop N demands were not fully met. In this study, the NNI of sunny maize MCS with or without N and of shaded maize CFS without N were lower than 1 (Table 3), whereas the NNI of CFS with N was higher than 1. It means that the application of 135 kg N ha-¹ was insufficient to overcome the theoretical N limitation of sunny MCS (Table 3), but it was sufficient for shaded CFS.

The increases in NNI from 0.26 (without N) to 0.45 (with N) (Tab. 3) were respectively accompanied by increases in crop production from 1272 kg ha-¹ to 8044 kg ha-¹ (Fig. 3).

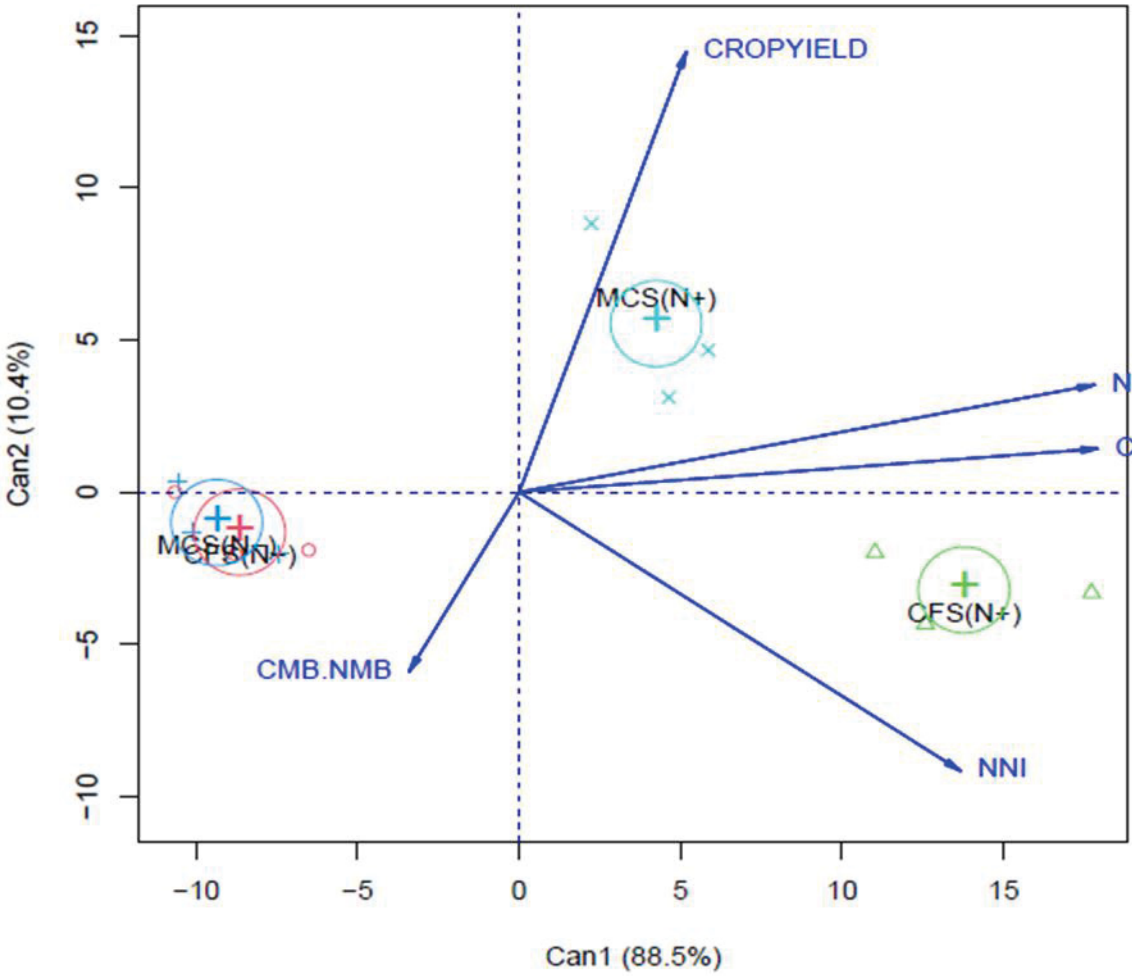
Figure 3 – Maize crop yield under Monoculture Crop and Crop-Forestry systems and two N rates. N- (without N fertilization) and N+ (with the application of 135 kg N ha-1 when maize had three leaves fully expanded) in a seven-year-old integrated agricultural system in Pinhais, Paraná, Brazil, in the cropping season of 2019-2020.



*Interpretation: Means followed by different small letters compare N fertilization in the systems, and capital letters highlight the differences between systems in N fertilization treatments, according to the Tukey test at $p<0.05$

We also analyzed the data through a canonical correlation analysis (CCA) (Fig. 4). The first pair of canonical variables explained 98.9% of the observed variability. The canonical correlations confirmed that CMB, NMB, and grain yield were the variables that most influenced the MCS treatment, particularly when N was applied. Along these lines, the areas without N were strongly described by the decreases in the CMB:NMB ratios. However, it is interesting to notice that the CCA also showed that the NNI of N-fertilized maize was associated with the CFS, which was considered to be a shaded system.

Figure 4 - Canonical correlation analysis (CCA) between treatments (MCS and CFS) and soil attributes (CMB, NMB, crop yield, NNI, and CMB:NMB) in the seven-month long-term experiment in Pinhais, Paraná, Brazil, from September 2019 to March 2020. * The graph shows that crop yield, CMB, and NMB were associated with MCS and increased in the same direction in the same quadrant, meaning that they are highly correlated with each other. On the other hand, the NNI increased in the opposite direction to the indicators above in the CFS.



*Legend: MCS = monoculture crop system; CFS = crop-forestry system; CMB = C-microbial biomass; NMB = N-microbial biomass; crop yield: maize grain yield; NNI = Nitrogen Nutrition Index; CMB:NMB = ratio of CMB to NMB; MCS(N-) = crop system without N fertilization; CFS(N-) = crop-forest system without N fertilization; MCS (N+) = monoculture crop system with N fertilization; and CFS (N+): crop-forestry system with N fertilization. Triangles, circles, multiplication, and plus signs indicate the distribution of the means of the treatments. × indicates MCS (N+); ∆ indicates CFS (N+); + indicates MCS (N-), and ° indicates CFS (N-).

Interpretation: Vectors inside the positive quadrants demonstrate positive correlations with the analyzed variables, while vectors inside the negative quadrants indicate negative correlations with the analyzed variables.

4 Discussion

4.1 CMB and NMB as indicators of soil quality in integrated CLFS

The CMB and NMB measurements are among the most recommended microbiological indicators to show the influence of soil use changes on its biogeochemical dynamics (Kaschuk et al., 2010; Lopes et al., 2018; Anzalone et al., 2020; Valani et al., 2020; Lazeris et al., 2021; Ferreira et al., 2021). Soil microbial biomass plays central roles in nutrient cycling, soil suppressiveness of plant diseases, biological control, and soil and water detoxication (pollutant degradation), and it is often related to increased yields as it behaves as a reservoir of soil nutrients (Kaschuk et al., 2010; Franzluebbers, 2018, 2020; Anzalone et al., 2020; Ferreira et al., 2021). Determining C and N in microbial biomass gives insight into more efficient and sustainable agricultural systems. For example, considering 111 maize crop fields in different locations in the USA, Franzluebbers (2018, 2020) demonstrated in a series of studies that increased soil N availability for plant uptake varied among different types of farm management and was closely related to the soil biological activity, probably due to increased net N mineralization provided by soil microbial biomass.

In this study, the values of CMB varied from 316 to 436 mg C kg^{-1} soil (Table 2), which are considered satisfactory from the point of view of a soil quality indicator (i.e., well above critical values of 170 mg C kg^{-1}; Anzalone et al., 2020); nevertheless, they varied between different agricultural systems (Table 2). The explanations for the CMB satisfactory values may be related to the fact that the plots of this long-term experiment were always cultivated without soil turnover, which contributed to soil organic matter accumulation and CMB growth over time (Kaschuk et al., 2010; Anzalone et al., 2020). Moreover, the four agricultural systems were cultivated with grasses, which are known for abundant roots that deposit soluble exudates and dead root fragments, increasing soil C supply and microbial growth (Moraes et al., 2014, 2019; Paes et al., 2018; Bieluczyk et al., 2020; Gamboa et al., 2020). Furthermore, satisfactory values of CMB across different agricultural systems, including the intensive MCS, are attributed to the fact that these integrated agricultural systems are being managed without the input of biocides, which could be comparable to organic farming systems during the experiment setup, improving soil fertility, particularly regarding K+, Mg+, and Ca+ contents (Table 1), and altogether promoting soil microbial growth (Kaschuk et al., 2010; Ferreira et al., 2021).

The addition of organic matter through the use of cover crops can increase microbial activity, mainly due to heterotrophic microorganisms, which have increased activity to obtain C, N, P, and S (Bell et al., 2006), which together with humic, non-humic, and soluble substances are used as a primary source of energy for microbial biomass (Sekaran et al., 2021). The process occurs through the release of organic substances from the roots, which function as a source of energy for heterotrophic microorganisms, and which, together with sugars and amino acids, stimulate the production of hydrolyzing enzymatic compounds which release nutrients to the soil, thus increasing the activity of microbial biomass (Sekaran et al., 2021). Thus, the differences in CMB between CLS and CFS are probably related to these organic compounds released by the roots, which means that there is a greater amount of biological substrates for the growth of the microbial population (Faissal et al., 2017; Sekaran et al., 2021). In this case, the CMB differences between grazed and ungrazed areas may be temporary adjustments to the microbial community structure.

The values of NMB and the CMB:NMB ratios were affected by the presence of livestock in CLS and CLFS (Table 2), probably due to increases in N availability caused by the deposition of N via urine (Tedesco et al., 2004; Moraes et al., 2014; 2019) and by N fertilization (Table 2). These results suggest that soil N availability limited microbial biomass in these integrated agricultural systems. When the N is applied as fertilizer, about 2–5% of the N remains incorporated into the microbial biomass and is released after cell death (Batista et al., 2018; Ferreira et al., 2021). Thus, sustaining high values of CMB in the systems is an achievable strategy to improve nutrient recycling in the systems because the supply of extra N is transiently immobilized by soil microbial biomass but is made available later (Kaschuk et al., 2010; Anzalone et al., 2020).

In this study, treatments affected CMB:NMB ratios. However, it is interesting to mention that different species of microbial biomass maintain stoichiometric proportions of nutrients in their cells (Spohn and Chodak, 2015; Lazeris et al., 2021; Ferreira et al., 2021), and changes in the availability of nutrients in the soil must incur changes in the structure of the soil microbial community (Zhang et al., 2018). In this context, the presence of animals in the CLS relative to the CFS may be closely related to the decrease of the CMB:NMB ratios (i.e., higher NMB in the CLS and

CFS), possibly caused by the acceleration of waste decomposition, by grazing, and the addition of animal excreta, which stimulate root exudates (Sekaran et al., 2021). These changes in soil microbial structure, such as changes in the fungus:bacteria ratio in soil microbial biomass (Aleixo et al., 2014; Zhang et al., 2018), stimulation of soil microbial activity (Franzluebbers, 2018; 2020; Ferreira et al., 2021), and/or soil microbial renewal (Ferreira et al., 2021), may have increased N immobilization and consequently NMB (Sekaran et al., 2021).

4.2 Relationships between CMB, NMB, NNI and maize yield in shaded CFS

In the second part of the study, we verified the hypothesis that increases in CMB and NMB result in increased plant N nutrition and crop yields. This hypothesis agrees with data obtained by Franzluebbers (2018, 2020), who emphasized that improved soil health leads to concomitant increases in soil microbial activity, nutrient availability, and crop yields. Considering the MCS and CFS together, the N fertilization significantly increased CMB, NMB, NNI, and crop yields (Table 3 and Fig. 3), which may have confirmed our hypothesis. However, taking MCS and CFS separately, the results indicate that N fertilization has different effects on crop yields depending on the availability of sunlight (Figs. 3 and 4). For this part of the study, we only considered the MCS and CFS as they were cultivated with maize during the analyses. The presence or absence of eucalyptus trees distinguishes the MCS and CFS. Trees reduce the area’s light interception capacity (Karvatte et al., 2016; Pontes et al., 2018; Krulcheski et al., 2021) as well as the N assimilation routes (Fu, 2017; Liang, 2020; Lu, 2013; Wei, 2018, Geremia et al., 2018). Thus, due to changes in light availability, the maize crop receiving N fertilization presented a higher NNI in the CFS than in the MCS (Table 3; Fig. 4); however, it had a 61% lower grain yield (Fig. 3). Indeed, crop growth, development, and yield formation are all limited by N availability, and maize, like other crops, promptly responds to increased soil N availability (Du et al., 2020; Pico and Vyn, 2021; Pott et al., 2021; Ferreira et al., 2021); however, changes in plant N metabolism caused by shade result in lower crop yield.

We surmise that the CFS plants had to adjust their N concentrations to produce more photosynthetic pigments (chlorophyll) because they have about 32% less incident radiation than CS (Kruchelski et al., 2021) and were limited by light to produce more biomass (Karvatte et al., 2016; Pontes et al., 2018). Indeed, before sowing maize, the experimental area had accumulated 3.6 t ha^{-1} of dry matter crop residues in fully-sunny areas and 2.8 t ha^{-1} in areas integrated with trees (Table 1), evidencing a reduced potential for crop biomass production.

Increases in plant N concentration raise photosynthetic rates and produce higher crop yields (Mu and Chen, 2021). The NNI is one of the methods used to estimate the crop's nutritional status and relates to crop N use efficiency (Lemaire and Gastal, 1997; Lemaire et al., 2008; Gastal et al., 2015; Costa et al., 2017; Bernardon et al., 2020). NNI equal to 1 indicates that the crop's nutritional status of N is considered optimal, whereas NNI lower than 1 indicates that the crop is suffering from N deficiency, and NNI higher than 1 indicates that the crop is showing N luxury consumption and low N use efficiency (Gastal et al., 2015). In sunny MCS, increases in NNI are often positively correlated with grain yields (Du et al., 2020; Pico and Vyn, 2021; Pott et al., 2021); however, the NNI of shaded maize in the CFS receiving N was higher than 1; however, crop yields were low (Table 3), indicating that the application of N to shaded maize in the CFS resulted in theoretical N luxury consumption and consequently, a low N use efficiency. Historically, one of the most successful cases of farming intensification has been soil N fertilization, which can be achieved by the application of N-rich residues such as animal manure and N mineral fertilizers (Ferreira et al., 2021; Reznick et al., 2021; Rufino et al., 2021) and also by seed inoculation with plant growth-promoting diazotrophic bacteria (Kaschuk and Hungria, 2017; Kaschuk et al., 2022). However, the rates of N application for crops are usually determined in sunny monocropping systems in different edaphoclimatic conditions, and the recommendation Table is used within geopolitical maps (e.g., Raij et al., 1997; Tedesco et al., 2004; Motta and Pauletti, 2019) instead of regarding the particularities of individual farming systems (Franzluebbers, 2018; Franzluebbers, 2020). Thus, the results presented here show that Tables for N fertilization have to consider the distinct edaphoclimatic conditions of integrated CLFS to avoid excessive N fertilization rates. The light interception capacity and the edaphoclimatic changes of the system should be considered to increase both N use efficiency in the systems and agricultural sustainability under CFS due to forest plantations. Farmers could prune branches or increase the space between the trees under the CFS to improve the NNI.

5 Conclusions

Integrated agricultural systems promote soil quality, so the soil microbial biomass promptly responds to the addition of N and ensures more efficient N cycling in the soil ecosystem.

The results corroborate that adding N to the soil simultaneously increases CMB, NMB, NNI, and grain yields in sunny conditions. However, the usual rates of N fertilization recommended for sunny cropping systems do not agree with a model in which additional N will lead to concomitant increases in soil microbial biomass, NNI, and crop yields if applied in shaded cropping systems. More research is required to determine the most effective N fertilization rates in these systems.

Funding

This work was supported by the National Council for Scientific and Technological Development (CNPq grant numbers: 420140/2016-6 and 481646/2011-6). It also had educational support from the Federal University of Paraná Graduate Support Program (UFPR/PROAP, Curitiba, Brazil) and the Coordination for the Improvement of Higher Level Education Personnel (CAPES – Finance Code 001).

Acknowledgments

The authors acknowledge Heila de Araújo and Fabiana Gavelaki at the laboratories of the Department of Soils (UFPR, Curitiba, Brazil) and the staff at the Laboratory of Agroindustrial Quality of the Technological University of Paraná (UTFPR, Pato Branco) for their support during the analyses performed for this work, and the colleagues Tangriani Simionni Assmann, Patricia Mara de Almeida, Lya Bento Barbosa, Samia Rayara de Souza Ribeiro, Daniela Maria Martin, and Renata Francieli Moraes for heICLSing with sample preparation and data reading.

6 References

Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.D.M., Sparovek, G. (2013). Köppen's climate classification map for Brazil. Meteorologische Zeitschrift.22:6. doi: https://10.1127/0941-2948/2013/0507

Anzalone, R., Vezzani, F.M., Kaschuk, G., Hungria, M., Vargas, L., Nogueira, M.A. (2020). Establishing reference values for soil microbial biomass-C in agroecosystems in the Atlantic Forest Biome in Southern Brazil. Ecological Indicators, 117:106586. https://doi: 101016/j.ecolind.2020.106586

Aleixo, A.P., Kaschuk, G., Alberton O. (2014). Soil fungal and bacterial biomass determined by epifluorescence microscopy and mycorrhizal spore density in different sugarcane managements. Ciência Rural, 44:588-594. https://doi:10.1590/S0103-84782014000400002

Bernardon, A., Assmann, T.S., Soares, A.B., Franzluebbers A., Maccari M., Bortolli, M.A. (2020). Carryover of N-fertilization from maize to pasture in an integrated crop-livestock system. Archives Agronomy Soil Science, 66:1-16. doi: 10.1080/03650340.2020.1749268

Batista E.R., Zanchi C.S., Ferreira D.A., Santiago F.L.A., Pinto F.A., Santos J.V., Paulino H.B., Carneiro M.A.C. (2018). Atributos biológicos do solo em sistema integrado de produção agropecuária. In: Souza E.D., Silva F.D., Assmann T.S., Carneiro M.A.C., Carvalho P.C.F., Paulino H.B. (Eds). Sistemas Integrados de Produção Agropecuária no Brasil. Tubarão, SC. p.71-90.

Bremner, J.M. (1965). Total nitrogen. In: Black, C.A., (Eds). Methods of soil analysis Madison: American Society of Agronomy. Madison, W.I. p.1149-1178

Bieluczyk, W., Piccolo, M.C. Pereira, M.G., Moraes, M.T., Amin, S.A., Bernardi, A.C.C., Pezzopane, J.R.M., Oliveira, P.P.A, Moreira, M.Z., Camargo, P.B., Dias, T.S., Batista, I., Cherubin, M.R. (2020). Integrated farming systems influence soil organic matter dynamics in southeastern Brazil. Geoderma, v.371. https://doi:10.1016/j.geoderma.2020.114368

Bell, J.M., Robinson, C.A., Schwartz, R.C. (2006). Changes in soil properties and enzymatic activities following manure applications to a Rangeland. Rangeland Ecol. Manage., 56: 314-320. doi: https://doi.org/10.2111/05-172R1.1

Costa, N.D.L., Gianluppi, V., Moraes, A.,Carvalho, P.C.F., Magalhães, J.A.(2017). Nitrogen nutrition index of Trachypogon plumosus pastures in Roraima's savannas. Pubvet, 11:825-831. https://doi:10.22256/PUBVET.V11N8.825-831

CONAB (2020). Cost of Agricultural Production. Available online at: http://www.conab.gov.br/conab/Main.php?MagID=3&MagNo=39 (accessed November 30, 2022).

Carrer, M.J., Maia, A.G., Vinholis, M.M.B., Filho, H.M.S. (2020). Assessing the effectiveness of rural credit policy on the adoption of integrated crop-livestock systems in Brazil, Land Use Policy 92:104468. https://doi:10.1016/j.landusepol.2020.104468

Deiss, L., Kleina, G.B., Moraes, A., Franzluebbers, A.J., Motta, A.C.V., Dieckow, J., Sandini, I.E., Anghinoni, I., Carvalho, P.C.F. (2019). Soil chemical properties under no-tillage as affected by agricultural trophic complexity. European Journal Soil Science.171:1090-¹105. https://doi:10.1111/ejss.12869

Derpsch, R., and Friedrich, T. (2009). "Development and current status of no-till adoption in the world," in Proceedings on CD 18th Triennial Conference of the International Soil Tillage Research Organization (Izmir: ISTRO), 15–19.

Du, L., Li, Q,. Li, L., Wu, Y., Zhou, F., Liu, B., Zhao, B., Li, X., Liu, Q., Kong, F., Yuan, J. (2020) Construction of a critical nitrogen dilution curve for maize in Southwest China. Scientific Reports, 10:13084. https://doi:10.1038/s41598-020-70065-3

Embrapa (2020). Adoção de sistemas de integração lavoura-pecuária-floresta (IICLSF) em São Paulo. Boletim de Pesquisa e Desenvolvimento. ISSN: 1980-6841. 56p.

FAOSTAT (2023) Food and Agriculture Organization of the United Nations (FAO). FAOSTAT Database. Crops and livestock products. https://www.fao.org/faostat/en/#data/QCL

Faissal, A., Ouazzani, N., Parrado, J.R., Dary, M., Manyani, H., Morgado, B.R., Barragán, M.D., Mandi, L. (2017). Impact of fertilization by natural manure on the microbial quality of soil: Molecular approach. Saudi Journal of Biological Science, 24: 1437-¹443. https://10.1016/j.sjbs.2017.01.005

Feije, F., Anger, V. (1972). Spot test in inorganic analysis. Analytica Chimica Acta. 149: 363-367

Ferreira, G.W.D., Rau, B.M., Aubrey, D.P. (2021). Temporal nitrogen dynamics in intensively managed loblolly pine early stand development. Forest Ecology Management, 48:118890. https://doi: 10.1016/j.foreco.2020.118890

Franzluebbers, A. (2020). Soil-test biological activity with the flush of CO2: Validation of nitrogen prediction for maize production. Agronomy Journal, 112:2188-2204. https://doi: 10.1002/agj2.20094

Franzluebbers, A. (2018). Soil-test biological activity with the flush of CO2: III Maize yield responses to applied nitrogen. Soil Science Society of America Journal, 82:708-721. Htts://doi:10.2136/sssaj2018.01.0029

Fu, Y., Li. H.Y., Yu, J., Liu, H., Cao, Z., Manukovsky, N.S., Liu, H. (2017). Interaction effects of light intensity and nitrogen concentration on growth, photosynthetic characteristics and quality of lettuce (Lactuca sativa L. var. youmaicai). Scientia Horticulturae, 214:51-57. https://doi:10.1016/j.scienta.2016.11.020.

Gastal, F., Lemaire, G., Durand, J.L., Louarn, G. (2015). Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. Crop Physiology, 2:161-206. https://doi: 10.1016/B978-0-^{1}2-417104-600008-X

Geremia, E.V., Crestani, S., Mascheroni, J.D.C., Carnevalli, R.A., Mourão, G.B., Silva, S.C. (2018). Sward structure and herbage intake of Brachiaria brizantha cv Piatã in a crop-livestock-forestry integration area. Livestock Science, 212: 83-92. https://doi:10.1016/j.livsci.2018.03.020

Gonzalez-Quiñones, V., Stockdale, E.A., Banning, N.C., Hoyle, F.C., Sawada, Y., Wherrett, A.D., Murphy, D.V. (2011). Soil microbial biomass: Interpretation and consideration for soil monitoring. Soil Research, 49: 287-304.

Gamboa, C.H., Vezzani, F.M., Kaschuk, G., Favaretto, N., Cobos, J.Y.G., Costa, G.A. (2020). Soil-Root Dynamics in Maize-Beans-Eggplant Intercropping System under Organic Management in a Subtropical Region. Journal Soil Science and Plant Nutrition, 20:1480–1490. https://doi:10.1007/s42729-020-00227-9

Glatzle, S., Stuerz, S., Giese, M., Pereira, M., Almeida, R.G., Bungenstab, D.J., Macedo, M.C.M., Asch, F. (2021). Seasonal dynamics of soil moisture in an integrated-crop-livestock-forestry system in Central-West Brazil. Agriculture, 11:245. https://doi:10.3390/agriculture11030245

Haney, R.L., Franzluebbers, A.J., Porter, E.B., Hons, F.M., Zuberer, D.A. (2004). Soil carbon and nitrogen mineralization: Influence of drying temperature. Soil Science Society of America Journal, 68:489-492. doi: 10.2136/sssaj2004.4890

Himmelstein, J., Ares, A., Gallagher, D., and Myers, J. (2017). A meta-analysis of intercropping in Africa: impacts on crop yield, farmer income, and integrated pest management effects. Int. J. Agric. Sustain. 15, 1–10. doi: 10.1080/14735903.2016.1242332

Kaschuk, G., Alberton, O., Hungria, M. (2010). Three decades of soil microbial biomass studies in Brazilian ecosystems: Lessons learned about soil quality and indicators for improving sustainability. Soil Biol. Biochem. 42:1-13. https://doi.org/10.1016/j.soilbio.2009.08.020

Kaschuk, G., Hungria, M. (2017). Diversity and importance of diazotrophic bacteria to agricultural sustainability in the Tropics. In: de Azevedo J, Quecine M. (eds) Diversity and benefits of microorganisms from the tropics. p.269-298. https://doi: 10.1007/978-3-319-55804-2_12

Kaschuk, G., Galli-Terasawa, L.V., Albernas, K.K., Ripka, L.P., Pansolin, I.C., Matos, C.L., Pauletti, V., Kava, V. (2022). Inoculation of plant growth promoting Bacillus spp. in N-fertilized maize crop in soils with high organic matter content in South Brazil. Journal Agriculture Science, 14: 43-52 https://doi.org/10.5539/jas.v14n7p43

Kruchelski, S., Trautenmüller, J.W., Deiss, L., Trevisan, R., Cubbage, F., Porfírio-da-Silva, V., Moraes, A. (2021). Eucalyptus benthamii Maiden et Cambage growth and wood density in integrated crop-livestock systems. Agrofororesty System, 95: 1577–1588. https://doi:10.1007/s10457-021-00672-0

Karvatte, N., Klosowski, E.S., Almeida, R.G., Mesquita, E.E., Oliveira, C.C., Alves, F.V. (2016). Shading effect on microclimate and thermal comfort indexes in integrated crop-livestock-forest systems in the Brazilian Midwest. International Journal of Biometeorology, 60:1933–1941 https://doi: 10.1007/s00484-016-1180-5

Moraes, A., Carvalho, P.C.F., Crusciol, C.A.C., Lang, C.R., Pariz, C.M., Deiss, L., Sulc, M.R. (2019). Integrated Crop-Livestock Systems as a Solution Facing the Destruction of Pampa and Cerrado Biomes in South America by Intensive

Monoculture Systems. In: Agroecosystem Diversity: Reconciling Contemporary Agriculture and Environmental Quality. Elsevier: Amsterdam, The Netherlands (2019) p.257–273, https://doi: 10.1016/B978-0-12-811050-8.00016-9

Moraes, A., Carvalho, P.C.F., Anghinoni, I., Lustosa, S.B.C., Costa, S.E.V.G.A., Kunrath, T.R. (2014). Integrated crop–livestock systems in the Brazilian subtropics. European Journal of Agronomy, 57:4–9. https://doi:10.1016/j.eja.2013.10.004

Lu, D., Sun, X., Wang, X., Yan, F., Lu, W. (2013). Effect of shading during grain filling on the physicochemical properties of fresh waxy maize. Journal of Integrative Agriculture, 12:1560-1567. https://doi:10.1016/S2095-3119(13)60560-2.

Liang, X.G., Gao, Z., Shen, .S, Paul, M.J., Zhang, L., Zhao, X., Lin, S., Wu, G., Chen, X.M., Zhou, S.L. (2020). Differential ear growth of two maize varieties to shading in the field environment: Effects on whole plant carbon allocation and sugar starvation response. Journal of Plant Physiology, 251:153-194. https://doi:10.1016/j.jpICLSh.2020.153194

Lopes, A.A.C., Sousa, D.M.G., Reis Junior, F.B., Figueiredo, C.C., Malaquias, J.V., Souza, L.M., Mendes, I.C. (2018). Temporal variation and critical limits of microbial indicators in oxisols in the Cerrado, Brazil. Geoderma, 12:72-82. https://doi:10.1016/j.geodrs.2018.01.003

Lazeris, T.S., Souza, J.P., Vezzani, F.M., Lima, C.M., Kaschuk, G. (2021). Carbon and phosphorus biogeochemical cycles in native forest and horticultural areas in the Metropolitan Region of Curitiba, Brazil. Colloquium Agrariae, 17:1-11. https://doi:10.5747/ca.2021

Lemaire, G., Jeuffroy, M.H., Gastal, F. (2008). Diagnosis tool for plant and crop N status in vegetative stage Theory and practices for crop N management. European Journal of Agronomy, 28:614-624. https://doi: 10.1016/j.eja.2008.01.005

Lemaire, G., Franzluebbers, A., Carvalho, P.C.F., Dedieu, B. (2014). Integrated crop–livestock systems: Strategies to achieve synergy between agricultural production and environmental quality. Agriculture Ecosystem Environment, 190:4-8. https://doi:10.1016/j.agee.2013.08.009

Lemaire, G., Gastal, F. (1997). N uptake and distribution in plant canopies. In: Lemaire G, (ed.). Diagnosis of the nitrogen status in crops. Heidelberg: Spring-Verlag. 239 p.

Mu, X., Chen, Y. (2021). The physiological response of photosynthesis to nitrogen deficiency. Plant Physiology Biochemstry,158:76-82. https://doi: 10.1016/j.plaphy.2020.11.019

Pacín, F., Oesterheld, M. (2014). In-farm diversity stabilizes return on capital in Argentine agro-ecosystems. Agriculture Systems, 124: 51–59. https://doi:10.1016/j.agsy.2013.10. 008.

Paes, L.S.D.O.P., Lima, M.R., Kaschuk, G., Vezzani, F.M. (2018). Root development as an indicator of soil sustainability of cropping systems in the Atlantic Forest Biome. Cultivos tropicales, 39: 7-14.

Perosa, B., Newto, P., Carrer, M.J. (2021). Access to information affects the adoption of integrated systems by farmers in Brazil. Land Use Policy, https://doi:10.1016/j.landusepol.2021.105459

Peterson, C.A., Deiss, L., Gaudin, A.C.M. (2020). Commercial integrated crop-livestock systems achieve comparable crop yields to specialized production systems: A meta-analysis. Plos One 15:0231840. https://doi:10.1371/journal.pone.0231840

Pauletti, V., Motta, A.C.V. (2019). Manual de adubação e calagem para o estado do Paraná. SBCS - Sociedade Brasileira de Ciência do Solo. Curitiba-PR. 289 p.

Pariz, C. M., Costa, C., Crusciol, C. A. C., Castilhos, A. M., Meirelles, P. R. L., Roça, R. O., et al. (2017). Lamb production responses to grass grazing in a companion crop system with maize silage and oversowing of yellow oat in a tropical region. Agriculture Systems 151, 1–11. doi: 10.1016/j.agsy.2016.11.004

Pezzopane, J., Bonani, W., Bosi, C., Fernandes, E.R., Campos, B.A., Oliveira, P., Pedroso, F.A. (2020). Reducing competition in a crop–livestock–forest integrated system by thNNIing eucalyptus trees. Experimental Agriculture, 56:574-586. https://doi:10.1017/S0014479720000162

Pontes, L.S., Tullio, G.F., Martins, A.S., Moletta, J.L., Silva, V.P. (2018). Maize yield for silage and grains in different integrated crop-livestock systems. Revista Ciência Agronômica, 49:315-323. https://doi: 10.5935/1806-669020180036

Pott, L.P., Amando, T.J.C., Schwalbert, R.A., Gebert, F.H., Reimche, G.B., Pes, L.S., Ciampitti, I.A. (2021). Effect of hairy vetch cover crop on maize nitrogen supply and productivity at varying yield environments in Southern Brazil. Science of The Total Environment, 759:144313. https://doi: 10.1016/j.scitotenv.2020.144313

Pico, L.B.O., Vyn, T. (2021). Dry matter gains in maize kernels are dependent on their nitrogen accumulation rates and duration during grain filling. Plants-Basel, 15:10(6):1222. https://doi: 10.3390/plants10061222

Rufino, M.C., Gachene, C.K.K., Rodrigue, V.C.D., Hawkins, J., Onyango, A.A., Sanogo, O.M., Wanyama, I., Yesuf, G., Pelster, D.E. (2021). Sustainable development of crop-livestock farms in África. Frontiers of Agricultural Science and Engineering, 8:175–181. https://doi: 10.15302/J-FASE-2020362

Raij, B.V., Cantarella, H., Quaggio, J.A., Furlani, A.M.C. (1997). Recomendações de adubação e calagem para o Estado de São Paulo. Campinas, SP. Instituto Agronômico/ Fundação IAC. 285p

Reznick. J.P.K., Barth, G., Kaschuk, G., Pauletti, V. (2021). Nitrogen and cultivars as field strategies to improve the nutritional status of wheat grain and flour. Journal of Cereal Science, 102:103290. https://doi: 10.1016/j.jcs.2021.103290

Sekaran, U., Lai, L., Ussiri, D.A., Kumar, S., Clay, S. (2021). Role of integrated crop-livestock systems in improving agriculture production and addressing food security–A review. Journal of Agriculture and Food Research, 5:100190. https://doi: 10.1016/j.jafr.2021.100190

Sarto, M.V.M., Borges, W.L.B., Sartom J.R.W., Rice, C.W., Rosolem, C.A. (2020). Root and shoot interactions in a tropical integrated crop–livestock–forest system. Agricultural Systems, 181:102796. https: doi://101016/j.agsy2020.102796

Spohn, M., Chodak, M. (2015). Microbial respiration per unit biomass increases with carbon-to-nutrient ratios in forest soils. Soil Biololgy and Biochemistry, 81:128-133. https://doi:2015EGUGA..17.3838S

Santos, H.G., Jacomine, P.K.T., Anjos, L.H.C., Oliveira, V.A., Lumbreras, J.F., Coelho, M.R., Almeida, J.A., Filho, J.C.A., Oliveira, J.B., Cunha, T.J.F. (2018). Sistema brasileiro de classificação de solos. Brasilia, DF: Embrapa (2018). 355p.

Schroeder, J., Kammann, L., Helfrich, M., Tebbe, C.C., Poeplau, C. (2021). Impact of common sample pre-treatments on key soil microbial properties. Soil Biology and Biochemestry, 160:108321. https://doi: 10.1016/j.soilbio.2021.108321

Souza-Jr, C.M., Shimbo, J.Z., Rosa, M.R., Parente, L.L., Alencar, A., Rudorff, B.F.T., Hasenack, H., Matsumoto, M., Ferreira, L.G., Souza-Filho, P.W.M., Oliveira, S.W., Rocha, W.F., Fonseca, A.V., Marques, C.B., Diniz, C.G., Costa, D., Monteiro, D., Rosa, E.R., Vélez-Martin, E., Weber, E.J., Lenti, F.E.B., Paternost, F.F., Pareyn, F.G.C., Siqueira, J.V., Viera, J.L., Neto, L.C.F., Saraiva, M.M., Sales, M.H., Salgado, M.P.G., Vasconcelos, R., Galano, S., Mesquita, V.V., Azevedo, T. (2020). Reconstructing three decades of land use and land cover changes in Brazilian biomes with Landsat. Archive and Earth Engine. Remote Sensing, 12:2735, https://doi:10.3390/rs12172735

Sampaio, E.V.S.B., Salcedo, I.H., Maia, L.C. (1986). Limitações no cálculo da biomassa microbiana determinada pelo método da fumigação em solos com adição recente de substrato orgânico (14C). Revista Brasileira de Ciência do Solo. (1986) 10:31-35.

Santos, M.V., da Fonseca, D.M., Silva, L.D., Souza, W.F., Oliveira, T.S., Ferreira, L., Paciullo, D.S.C. (2020). Integrated crop–forage–forestry for sustainable agricultural systems: productive performance. Agroforestry Systems, 94:417–427. https://doi:10.1007/s10457-019-00404-5

Tedesco, M.J., Gianello, C., Bissani, C.A., Bohnen, H., Volkweiss, S.J. (1995). Análises de solo, plantas e outros materiais. Porto Alegre, Universidade Federal do Rio grande do Sul. 174p.

Tedesco, M.J., Gianello, C., Anghinoni, I., Bissani, C.A., Camargo, F.A.O., Wiethölter, S. (2004). Manual de adubação e de calagem para os Estados do Rio Grande do Sul e de Santa Catarina. Sociedade Brasileira de Ciência do Solo Comissão de Química e Fertilidade do Solo. Porto Alegre, RS. 376 p.

R Core Team. R: A language and environment for statistical computing. (2022). Vienna, Austria, R Foundation for Statistical Computing. Versão 4.0.2.

USDA-United States Department of Agriculture: Soil survey staff Keys to soil taxonomy, USDA-Natural Resources Conservation Service, Washington, DC. (2014). 372p.

Valani, G.P., Martíni, A.F., Silva, L.F.S., Bovi, R.C., Cooper. M. (2020). Soil quality assessments in integrated crop–livestock–forest systems: A review. Soil Use and Management, 37:22-36, https://doi:10.1111/sum12667

Vance, E.D., Brookes. P.C., Jenkinson, D.S. (1987). An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemestry, 9:703-707. https://doi:10.1016/0038-0717(87)90052-6

Van Keulen, H., Schiere, H. (2004). Crop–livestock systems: old wine in new bottles? In: New directions for a diverse planet. Brisbane, Australia. 12p.

Zhang, T., Chen, H.Y.H., Ruan, H. (2018). Global negative effects of nitrogen deposition on soil microbe. The ISME Jounral. 12:1817–1825. https://doi:10.1038/s41396-018-0096-y

Wei, H., Zhu, Y., Qiu, S., Han, C., Hu, L., Xu, D., Zhou, N., Xing, Z., Hu, Y., Cui, P., Dai, Q., Zhang, H. (2018). Combined effect of shading time and nitrogen level on grain filling and grain quality in japonica super rice. Journal of Integrative Agriculture, 7: 2405-2417. https://doi:10.1016/S2095-3119(18)62025-83

CHAPTER 3

NUTRIENT TRANSFER: THE EFFECTS OF NITROGEN FERTILIZATION ON THE TRANSITION FROM WINTER PASTURE TO MAIZE IN ICLS

NUTRIENT TRANSFER: THE EFFECTS OF NITROGEN FERTILIZATION ON THE TRANSITION FROM WINTER PASTURE TO MAIZE IN ICLS

ABSTRACT

We investigated the interaction between animals, plants and soil microbial biomass in Crop-Livestock Systems (ICLS) and their impact on nutrient cycling and grain productivity. ICLS have been recognized as a promising approach to improving soil quality and maximizing production efficiency. In this context, animals have stood out for their contribution to the sustainability and intensification of agriculture, including nutrient cycling. This study fills this gap by analyzing the influence of the presence of animals on soil microbial biomass in ICLS. The results show that the presence of animals improves soil nutrition and fertility, due to the constant cycling of nutrients between the soil-plant-animal system. In addition, the presence of animals promotes the accumulation of organic matter in the soil, favouring microbial activity and the processes of mineralization and transformation of nutrients. We hypothesize that the interaction between animals, plants and soil microbial biomass promotes a more dynamic and balanced nutrient cycle, resulting in higher soil biological quality and grain productivity. The Nitrogen Nutrition Index (NNI) and the availability of ammonium (NH_4^+) were evaluated, showing that nitrogen fertilization influences the absorption of nutrients by plants. However, the presence of animals can compensate for the lack of nitrogen fertilizers, promoting efficient nitrogen absorption. The results highlight the importance of soil microbial biomass in cycling nutrients and promoting soil health. Appropriate management strategies, such as the type of grazing and fertilizer application, are essential to optimize nutrient uptake by plants and minimize losses. These findings contribute to the development of more sustainable and efficient agricultural practices in ICLS.

1 Introduction

Integrated Crop-Livestock Systems (ICLS) can modify the soil's physical and chemical properties, improving soil quality, processes ranging from nutrient cycling to maximizing productivity (Hartmann et al., 2015; Zhou et al., 2014). Among the components included in ICLS, trees stand out due to the sustainable and intensified agriculture they provide, climate regulation, which guarantees animal comfort in grazing areas, biological pest control and nutrient cycling that can be optimized in these systems (Kruschelski et al., 2022).

Among the models proposed by ICLS, the integration of trees with grain-producing crops has been little explored. In a previous study, Ruthes et al., 2022 investigated the dynamics of the Nitrogen Nutrition Index (NNI) and the Carbon and Nitrogen of the microbial biomass (CMB and NMB, respectively) and concluded that in these areas the nitrogen applied exceeded the absorption capacity of the plants intended for grain production. This result highlights the complexity that ICLS can present.

One of the principles underpNNIing the premises of ICLS is the simultaneous or sequential exploitation of activities within the same area (Moraes et al., 2019). Among these activities is animal husbandry, which has been highlighted in several recent studies (Moraes et al., 2014; Martins et al., 2016; Adetunki etal., 2020; Alves et al., 2019), due to the improvement in soil fertility and the increase in some nutrients that are essential for plants, such as C and N (Cotrufo et al., 2013 ; Yu et al., 2014 ; Zhu et al., 2012).

The presence of animals in areas previously focused exclusively on grain production improves soil nutrition and fertility, due to the constant cycling of nutrients between the soil-plant-animal system (Assmann et al., 2017; Damian et al., 2021), as well as providing greater accumulation of organic matter, favouring soil microbial activity (Cavicchioli et al., 2019), ensuring the processes of mineralization and transformation of nutrients in the soil, with a direct effect on crop productivity (Stefan et al., 2021).

From this perspective, studies that explore nutrient cycling processes in ICLS and their impact on soil microbial activity play a fundamental role in advancing scientific knowledge and the search for more sustainable and efficient agricultural practices, since rotating pastures with grain production in the same area allows nutrients to cycle quickly, reducing the losses that would occur in fallow periods (Maccari et al., 2021) and tend to favor the activity of microbial biomass.

Some previous studies (Assmann et al., 2003; Balbinot Junior et al., 2011; Sandini et al., 2011) had already pointed out that fertilizing pastures could lead to the transfer of nutrients, especially N, to the following crop. However, there are few studies that delve deeper into understanding what actually happens to the soil's microbial biomass and the transfer of nutrients to successor crops. Although the fundamental role of microbial biomass in nutrient cycling and promoting soil health is recognized, there are still gaps in knowledge about the specific mechanisms involved in this process.

In this sense, this study hypothesized that the presence of animals increases the activity of soil microbial biomass (SBM) and the availability of ammonium (NH_4^+), which in turn favors the efficient transfer of nutrients to successor crops. We assume that the interaction between animals, plants and soil microbial biomass promotes a more dynamic and balanced nutrient cycle, resulting in a positive response in terms of soil biological quality and grain productivity.

The main objective was to understand how this interaction between animals, plants and soil influences the competition for nitrogen between the plant and the soil's microbial biomass, especially in areas without the application of nitrogen fertilizers.

2 Material and methods

2.1 Site descripton

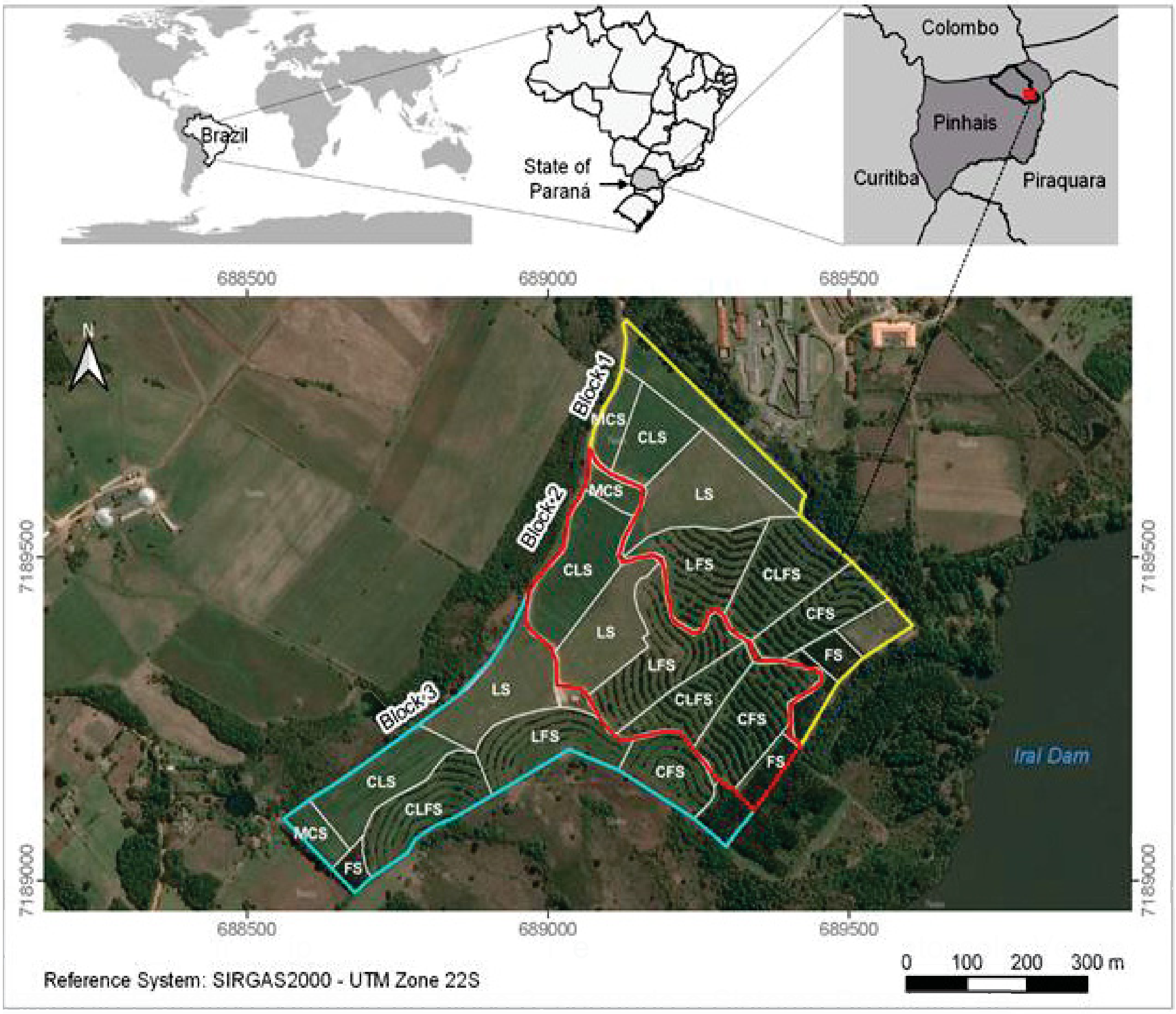
The experiments were carried out in two locations, in the municipality of Pinhais at the Canguiri Farm belonging to the Federal University of Paraná (UFPR) and in Candói at the Capão Redondo Farm, both in the state of Paraná, in areas of crop-livestock integration and maize farming. The climate of both locations is Humid Temperate with Temperate Summer (Cfb, Köppen) with no dry season and frost in winter (IAPAR, 2019)

2.2 History of the area

The experiment, located in the municipality of Pinhais, PR, was carried out as part of a long-term experiment which began in 2012 at the Núcleo de Inovação Tecnológica em Agropecuária (NITA). That year, the experimental area was ploughed with a scarifier to a depth of 40 cm and fertilized with 10 $Mg.ha^{-1}$ of N VIRO® which was applied to the surface of the soil (Kruchelski et al., 2021). In the winter of the same year, black oats (Avena strigosa) were sown with 100 kg.ha-¹ of P_2O_5 in the sowing line.

The NITA treatments were set up in randomized blocks with three replications and seven farming systems: crop, forest, livestock, crop-forest, crop-livestock, crop-livestock-forest and livestock-forest (Figure 1). Since then, soil fertilization has been carried out in a similar way in all systems, according to soil analyses and crop and pasture recommendations, according to Pauletti and Motta (2019). The chemical analysis of the soil before the experiment was set up is shown in Table 1

Figure 1 Experimental area at the Federal University of Paraná, municipality of Pinhais, Paraná, Brazil, and the treatments: monoculture system (MCS), livestock system (LS), forest (FS), crop-forest system (CFS), livestock-forest system (LFS), crop-livestock system (CLS) and crop-livestock-forest system (CLFS).



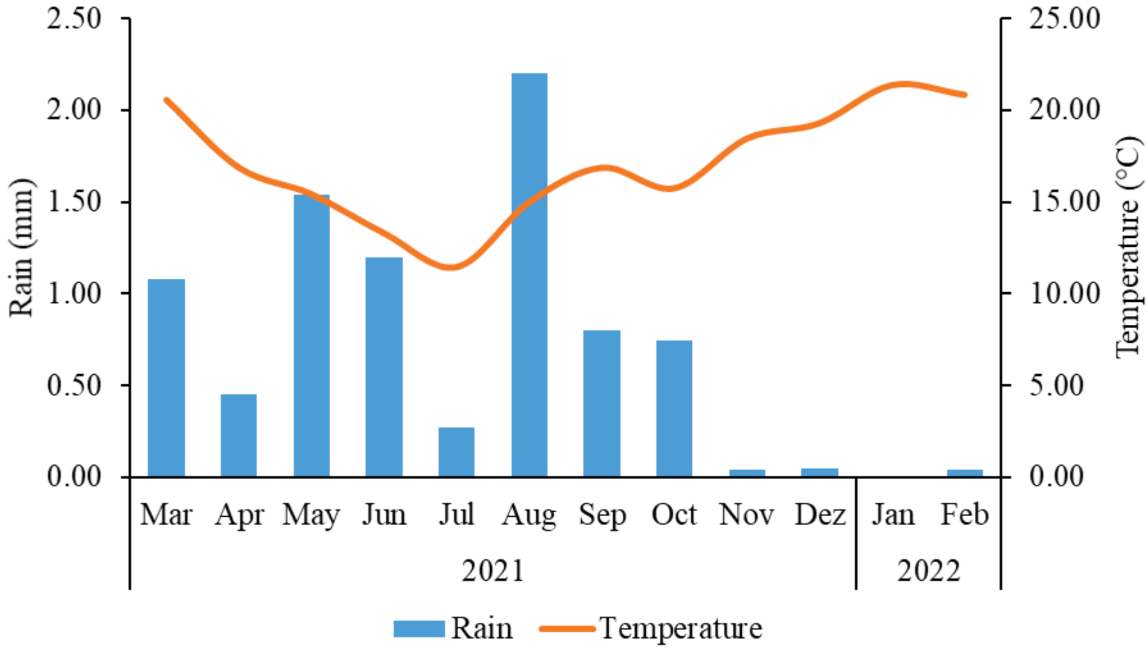
The soil in the current study's experimental area was classified as oxisols (USDA Classification System, 2014), or as typical dystrophic red-yellow latosol in the blocks 1 and 2 (Fig. 1), and as cambic eutrophic red-yellow latosol in the block 3, according to the Brazilian Classification System (Santos et al., 2018).

Table 1 Soil chemical attributes (average n=3) at 0-10 cm depth in the long-term experiment of different integrated agricultural systems at the Núcleo de Inovação Tecnológica em Agropecuáira (NITA), in Pinhais, Paraná, Brazil, in July 2021.

System#	pH	P	Organic matter	K^+	Ca^{2+}	Mg^{2+}	H+Al	SB	CEC	Crop residues
	$CaCl_2$	mg dm^{-3}	g dm^{-3}	--------cmol dm^{-3}---------						t ha^{-1}
Monoculture system	5.01	11.10	47.9	0.63	5.70	2.88	4.30	9.20	12.20	2.75
Crop-livestock system	5.02	8.50	45.60	0.58	5.07	2.57	3.57	8.50	12.00	2.69

SB = sum of bases. CEC = cation exchange capacity. The data was obtained according to the methodology proposed by Santos et al. (2018). In comparison with the guidelines of Pauletti and Motta (2018).

FIGURE 2 – Meteorological data from automatic weather stations in the NITA from March 2021 to February 2022.



Source: The autor (2023)

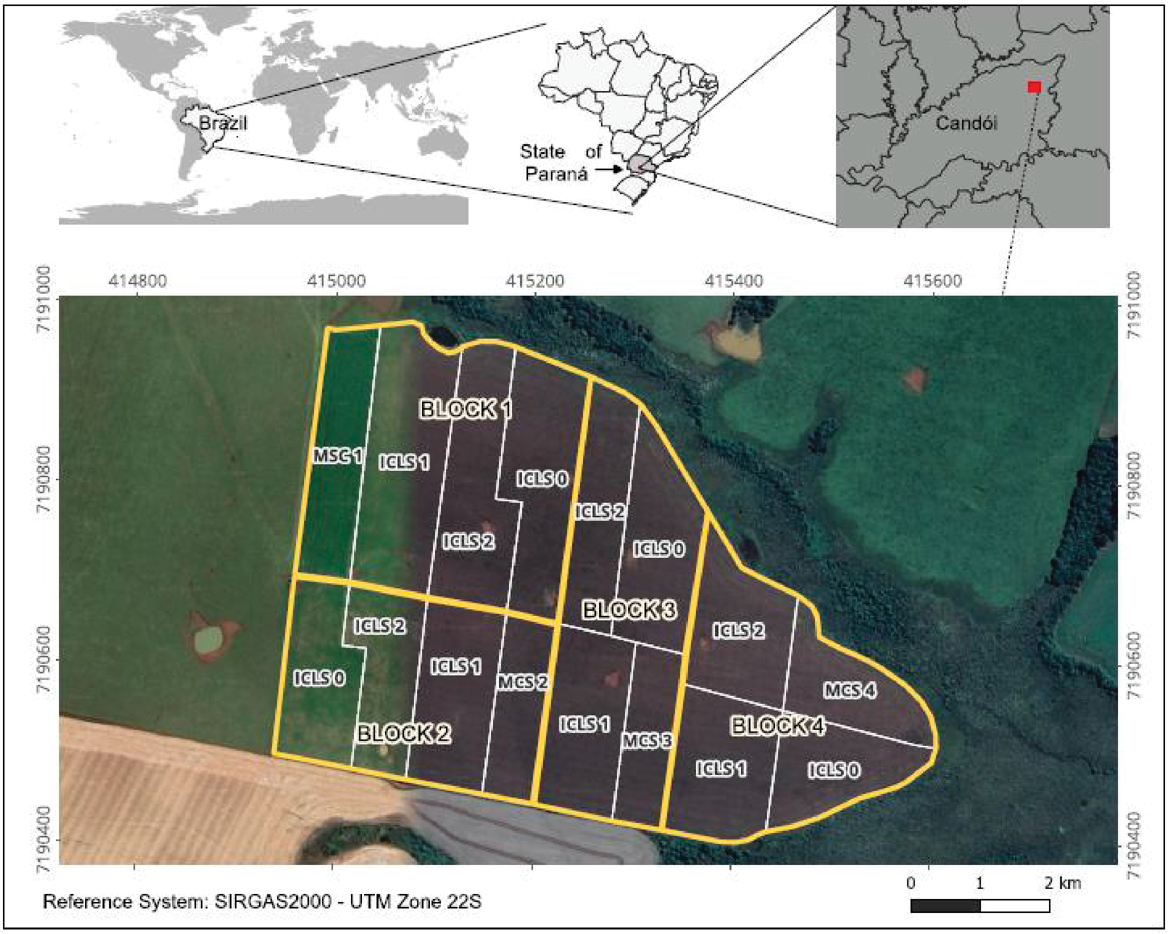
The experiment carried out at Fazenda Capão Redondo was installed in 2021 on an area of approximately 25 ha (Figure 3). Prior to setting up the experiment, the area was used for planting oats for grazing. In mid-June/2021, the experiment was set up in a randomized block design, with four replications. The treatments consisted of farming systems, which differed in terms of the duration of grazing during crop rotation, and the farming system, which did not include grazing: ICLS 1: with perennial pasture for one year, ICLS 2: perennial pasture for two years, ICLS 0: annual pasture during the winter and MSC: traditional agriculture consisting of cover crops during the winter and grain crops during the summer. Grazing took place on a rotational basis, lasting approximately 40 days. The chemical analysis of the soil before the experiment was set up is shown in Table 2.

Table 2 Soil chemical attributes (average n=3) at 0-10 cm depth at Fazenda Capão Redondo, Candói, Paraná, Brazil in July 2021.

System#	**pH**	**P**	**Organic matter**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**SB**	**CEC**	**Crop residues**
	$CaCl_2$	mg dm^{-3}	g kg	--------cmol dm^{-3}---------					t ha^{-1}
Total area	5.92	8.43	53.61	0.23	7.29	3.43	10.95	15.58	2.54

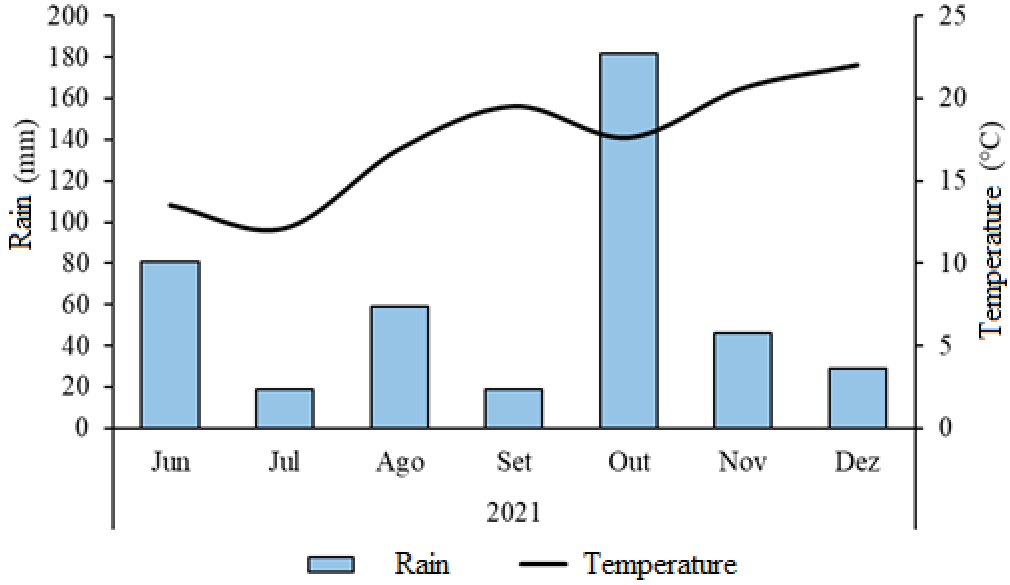
SB = sum of bases. CEC = cation exchange capacity. The data was obtained according to the methodology proposed by Santos et al. (2018). In comparison with the guidelines of Pauletti and Motta (2018).

Figure 3 Experimental area at Fazenda Capão Redondo, in the municipality of Candói, Paraná, Brazil, and the treatments: ICLS 1: with perennial pasture for one year, ICLS 2: perennial pasture for two years, ICLS 0: annual pasture during the winter and MSC: traditional agriculture which consisted of cover crops during the winter and grain crops during the summer.



The soil in the experimental area was classified as Latossolo Bruno alumínico típico associated with a Cambissolo, according to the Brazilian Classification System (Santos et al, 2018).

FIGURE 4 – Meteorological data from automatic weather stations in the Fazenda Capão Redondo from June 2019 to Dezember 2021



Source: The autor (2023).

2.3 Nitrogen fertilization

In the NITA area, nitrogen fertilization took place only as a top dressing, with 90 kg N ha-¹ being applied in the form of urea (45% N) (N+), 28 days after sowing the black oats. The maize crop was also fertilized as a cover crop, with 180 kg N ha-¹ applied in the form of urea (45% N) (N+), when the crop was at stage V3. Part of the area, around 50 m², received no nitrogen fertilization (N-).

At Fazenda Capão Redondo, the winter pasture was made up of a mixture of naturally reseeded ryegrass, over-seeded with 40 kg ha-¹ of Temprano rye. When sowing the rye, 180 kg ha-¹ of Monoammonium Phosphate - MAP 11-52-00 - was applied to all the plots, i.e. 19.8 kg ha-¹ of N and 93.6 kg ha-¹ of P_2O_5. Before planting the maize crop, the area was desiccated with Glyphosate 2 L ha-¹, Poker 0.8 ha-¹, Match 0.15 ha-¹ and Weicit 0.2 L ha-¹. The maize was fertilized with 190 kg N ha-¹ in the form of Monoammonium Phosphate - MAP 11-52-00. During the maize crop cycle, Premium 0.15 L ha-¹, Reglone 2 L ha-¹, Weitcit 0.12 L ha-¹, Galil 0.4 L ha-¹, Purynex 1 L ha-¹, Rizospirillum 0.5 L ha-¹ and Pirolenhoso 0.5 L ha-¹ were applied.

2.4 NNI determination

2.4.1 NNI winter grasses

The nitrogen nutritional status - NNI of the winter grasses was assessed using aerial part biomass data. Collections began from the moment the aerial part biomass reached 1 MS ha-¹ until the start of flowering in the areas where the grasses were used for cover and, in the ICLS areas next to the biomass collection of the exclusion cages, in both evaluation sites.

2.4.2 Maize crop NNI

At NITA, they chose to grow maize using the AS1757PRO3 hybrid, which is an early variety with transgenic technology against insects, due to its location in an environmental preservation area. At Fazenda Capão Redondo, the cultivar was Agroeste 1757.

At both sites, the samples were taken at stages V6, V10, V12 and V14, where five plants were collected per linear meter in each treatment to determine dry matter, while the crop was still in the vegetative stage and had already reached 1 Mg ha-¹ of DM. Immediately after collection, the plants were taken to an oven with forced air circulation, at a temperature of 55 °C, until constant mass. The dry matter of the whole plant was ground in a Willey mill and the N concentration was determined using the method of Tedesco (1995).

To estimate NNI, we used the equations presented by Gastal et al. (2015), which establish the relationship between the current N content and the critical N content determined, according to the equation: NNI = (% Na)/(% Nc), where: % Na corresponds to the current percentage of N and % Nc to the critical N.

2.5 Maize crop yield

At NITA, the maize grain was harvested on March 9, 2022 from a 12 m² area in each sub-plot. The cobs were then threshed and weighed. The moisture content of the grains was corrected to 13%.

At Fazenda Capão Redondo, the yield samples were collected on March 24, 2022, in an area of around 20 m² in each sub-plot. The ears were also threshed, weighed and the moisture content corrected to 13%.

2.6 Soil chemical analysis

The chemical analyses were carried out on dry soil, sieved through a 2 mm mesh, according to Santos et al., 2018. The pH was measured on samples resuspended in 0.01 mol.L-¹ $CaCl_2$. Organic C was determined after wet oxidation with 0.0667 mol L-¹ $K_2Cr_2O_7$ and titration with 0.05 mol L-¹ Fe (NH4)2(SO4)2.6H2O, using diphenylamine as an indicator. Organic matter was obtained by multiplying organic C by 1.724. Available P and K extracted with Mehlich. O (0.05 mol L-¹ HCl + 0.0125 mol L-¹ H_2SO_4) and determined by spectrophotometry. The exchangeable Al^{+3}, Ca^{+2}, Mg^{+2} and Al^{+3} were extracted with KCl 1 mol L-¹ and determined by atomic absorption spectroscopy..

2.7 Microbial biomass carbon

CMB was determined using the extraction-fumigation method of Vance et al., 1987, with minor modifications. Approximately 20 g of dry soil were weighed, rewetted to 50% of field capacity with deionized water and fumigated with chloroform for 48 hours in a fumigation box attached to a vacuum pump for 48 hours, these were called "fumigated samples". During the same period, other soil subsamples (20 g) were rewetted and kept in the dark at room temperature, called "non-fumigated".

For carbon (C) extraction, the samples were given 50 mL of 0.5 M K2SO4 extractant solution and suspended at 175 rpm on a horizontal orbital shaker for 60 minutes. The suspensions were then centrifuged at 2500 rpm for 10 min and the extract was obtained by filtering through slow filtration paper. The amount of C extracted from the soil was determined by colorimetry in a spectrophotometer using a 0.5 mL aliquot of the extract. The difference in C determined the CMB found in the fumigated and non-fumigated samples, multiplied by the correction factor KC=0.41 (Sampaio et al., 1986).

2.8 Soil ammonium (NH_4^+)

Soil ammonium was determined using the Fenato methodology (Apha, 1995). Approximately 10 g of soil was weighed and 15 mL of 2M KCl extractant solution was added. The samples were shaken for 45 min and filtered through rapid filtration paper. The amount of NH_4^+ in the soil samples was quantified by colorimetry using 0.5 mL of the extract.

3 RESULTS

3.1 NNI, NH_4^+ and CMB for winter grasses

The NNI was used to assess the efficiency of N use in the systems evaluated. During the winter period, the MCS areas remained ungrazed, while the ICLS were grazed throughout the winter. This difference in management directly interfered with the NNI of the grasses in the NITA. As expected, right after nitrogen fertilization in the tillage areas, there was an increase in NNI values and, as the plant developed, this NNI decreased over time. In the ICLS areas, on the other hand, there was an increase in NNI after the grazing animals left (Table 3).

Table 3 Nutritional Nitrogen Index (NNI), Ammonium (NH_4^+) and Carbon of Microbial Biomass (CMB) in the long-term experiment of different integrated Núcleo de Inovação Tecnnológica em Agropecuária (NITA), in Pinhais, Paraná, Brazil, in the 2021/2022.

Indicator*	**NNI**		**NH_4^+** mg dm³of soil^{-1}	**CMB** mg kg^{-1}
Period§	MSC	ICLS		
1	0.85 aA	0.41 bB	223.77 a	202.66
2	0.75 aA	0.43bB	149.01 b	179.84
3	0.55 bA	0.42 bA	242.77 a	166.78
4	0.57 bB	1.24 aA	179.98 ab	197.57
MCS			208.22	213.83 a
ICLS			188.93	159.59 b
CV (%)	15.41		21.87	32.14
p-value at F-test				
System	*0.0000*		*0.2950*	*0.0439*
Period	*0.5015*		*0.0089*	*0.7187*
System ×Period	*0.0000*		*0.1607*	*0.4778*

Sampling was carried out when the crop-livestock and crop-livestock systems were cultivated with oats with the application of 90 kg N ha-1 in the form of urea.

§For MCS: collected at 57, 70, 85 and 98 days after sowing, and ICLS: 52, 62, 111 and 132 days after sowing.

* Averages followed by the same uppercase letters in the column and lowercase letters in the row do not differ according to Tukey's test at p<0.05.

The concentration of ammonium in the soil indicates the availability of nitrogen in the form of NH_4^+ for plants and microbial activity. NH_4^+ values varied between 0.85 and 0.57 mg dm³of soil^{-1} for the cropping system and between 0.42 and 1.24 mg dm³of soil^{-1} for the crop-livestock system. No significant differences were observed between the cropping systems in terms of NH_4^+ concentration. CMB, on the other hand, showed that there were significant differences between the cropping systems in relation to CMB. The cropping system showed higher average CMB values compared to the crop-livestock system, indicating greater microbial activity in the soil of the cropping system.

At Fazenda Capão Redondo, the evaluation of the NNI of the winter grasses (Table 4) in both evaluation periods and treatments revealed that the NNI values were close to 1. These results from both evaluation sites show that the applications of the N doses were sufficient to overcome the theoretical N limitation.

Table 4 Nitrogen Nutrition Index (NNI), Ammonium (NH_4^+) and Microbial Biomass Carbon (CMB) in the long-term experiment of different integrated agricultural systems at Fazenda Capão Redondo, Candói, Paraná, Brazil, in different harvests 2021/2022.

Indicator*	NNI	NH_4^+ mg dm³of soil^{-1}	CMB mg kg^{-1}
Period§			
1	0.91	173.91	129.89
2	0.85	220.69	151.59
System			
MCS	0.84	213.17	146.95
ICLS	0.93	181.43	134.52
CV (%)	23.31	44.40	18.42
p-value at F-test			
System	*0.44440*	*0.4824*	*0.3565*
Period	*0.54424*	*0.3065*	*0.1200*
System ×Period	*0.79577*	*0.2752*	*0.6169*

The samples were collected when the Crop and Crop-Livestock systems were cultivated with sorghum and rye 180 kg N ha-1.

§Samples were collected 85 and 98 days after sowing.

* Averages followed by the same uppercase letters in the column and lowercase letters in the row do not differ according to Tukey's test at p<0.05.

3.2 NNI, NH_4^+ and CMB of the maize crop

In the NITA, in the ICLS areas where nitrogen fertilizer (N+) was applied, the NNI increased with the accumulation of biomass from the aerial part of the maize crop, while in the areas without nitrogen (N-) it remained constant during the maize growth cycle. The same was seen for NH_4^+, which remained constant over time at both levels of nitrogen fertilization. CMB, on the other hand, varied during the maize growth period (Table 5).

Table 5 Nutritional Index Nitrogen (NNI), Ammonium (NH_4^+) and Carbons Microbial Biomass (CMB) in the long-term experiment of different integrated agricultural systems at the NITA, in Pinhais, Paraná, Brazil, with or without N application during the 2021/2022.

Indicator*	**NNI**		**NH_4^+** mg dm³of soil^{-1}		**CMB** mg kg^{-1}	
			Fertilizer§			
System	N-	N+	N-	N+	N-	N+
MCS						
V6	0.69 bA	1.00 aA	92.62 bA	220.9 aA	135.01 aA	182.05 aA
V10	0.58 bB	0.99 aA	94.55 bA	163.45 aAB	265.77 aA	218.87 aA
V12	0.54 bA	0.71 aA	96.48 aA	115.15 aB	113.50 bA	114.52 bA
V14	1.19 aA	0.61 aB	98.41 bA	253.8 aA	174.42 aA	212.80 aA
ICLS						
V6	0.89 aA	0.51 bB	93.58	115.15	216.23 aA	350.38 aA
V10	0.95 aA	0.87 abA	95.51	108.10	161.17 bA	178.60 aB
V12	0.73 aB	1.14 aA	97.47	126.90	194.30 aA	192.57 aB
V14	0.86 aB	1.24 aA	99.97	118.15	195.55 aA	159.63 aB
CV (%)	22.73		30.72		27.99	
p-value at F-test						
System	*0.0593*		*0.6836*		*0.0714*	
N fertilizer	*0.1533*		*0.0078*		*0.1008*	
Period	*0.0495*		*0.0118*		*0.2699*	
System x N fertilizer	*0.9584*		*0.0420*		*0.4361*	
System x Period	*0.0502*		*0.7986*		*0.0059*	
N fertilizer x Period	*0.0661*		*0.0226*		*0.1245*	
System × N fertilizer ×Period	*0.0000*		*0.0618*		*0.0000*	

Samples were taken when the MCS and ICLS were cultivated with maize. 180 kg N ha-1, in the form of urea, was applied to half of the plots on November 11, 2021, and samples of paired plants (with and without N application) were taken at 66, 75, 86 and 97 days after sowing.

§N+: with 180 kg N ha-1 fertilization and N-: with 45 kg N ha-1 fertilization.

*Averages followed by the same uppercase letters in the column and lowercase letters in the row do not differ according to Tukey's test at p<0.05.

At Fazenda Capão Redondo, on the other hand, the NNI values were >1, revealing that the doses of N applied at the site were sufficient for the full development of the crop (Table 6), in both evaluation systems, without a nitrogen deficiency occurring for the maize crop. However, there was a decrease in NH_4^+ and CMB values, indicating that the doses of N applied were insufficient to maintain the soil's reserves.

Table 6 Nitrogen Nutrition Index (NNI), Ammonium (NH_4^+) and Carbon Microbial Biomass (CMB) in the experiment of different integrated agricultural systems at Fazenda Capão Redondo, Candói, Paraná, Brazil, with or without N application during the 2021/2022 growing season.

Indicator*	NNI	NH_4^+ mg dm³of soil⁻¹		CMB mg kg⁻¹	
System					
Crop	1.02	206.72		299.99	
Crop-Livestock	1.08	219.14		297.65	
N fertilization					
N-	1.04				
N+	1.06				
Period		N-	N+	N-	N+
V6	0.99	158.72 aB	389.08 aA	317.95 aB	408.88 aA
V10	1.06	220.12 aA	288.10 bA	332.62 aA	358.21 aA
V12	0.99	157.33 aB	193.08 cA	367.22 aA	176.07 bB
V14	1.17	131.25 aA	168.72 cA	253.63 aA	175.94 bA
CV (%)	23.2	30.77		29.17	
p-value at F-test					
System	*0.3683*	*0.4519*		*0.9149*	
N fertilizer	*0.7074*	*0.0000*		*0.0000*	
Period	*0.1465*	*0.0000*		*0.0869*	
System x N Fertilizer	*0.5234*	*0.0824*		*0.7979*	
N Fertilizer x Period	*0.4293*	*0.9303*		*0.3158*	
Period X System	*0.1270*	*0.0000*		*0.0000*	
System × N fertilizer ×Period	*0.1720*	*0.1389*		*0.1656*	

Samples were taken when the MCS and ICLS were cultivated with maize. Nitrogen fertilization took place on November 11, 2021, and samples of paired plants (with and without N application) were collected at 66, 75, 86 and 97 days after sowing.

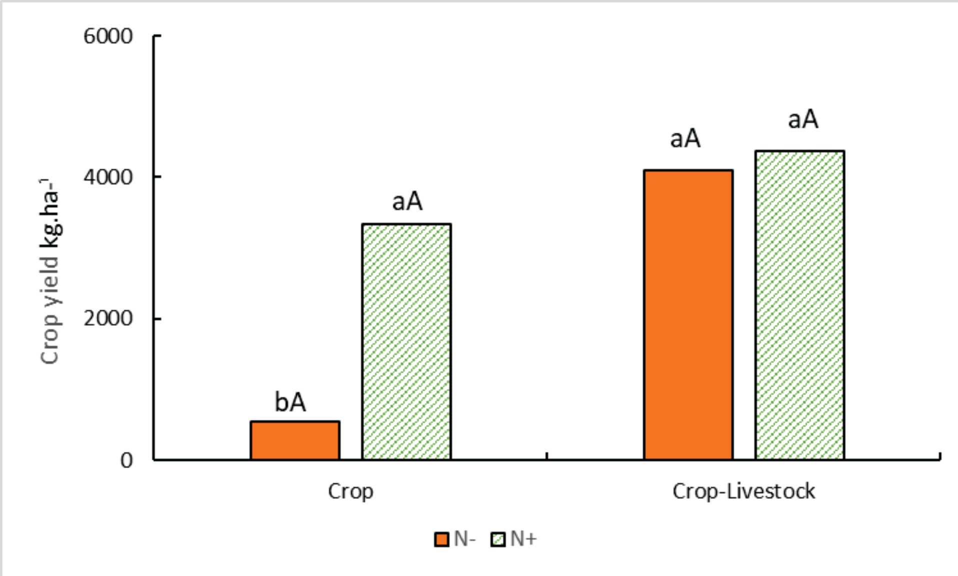
§N+: with fertilization of 190 kg N ha-1 in the form of Monoammonium Phosphate - MAP 11-52-00 in top dressing and N-: with fertilization of 15 kg N ha-1 also in the form of Monoammonium Phosphate - MAP 11-52-00.

*Averages followed by the same uppercase letters in the column and lowercase letters in the row do not differ according to Tukey's test at p<0.05.

3.3 Grain yield

The increase in NNI from 0.69 (N-) to 1.00 (N+) in the NITA tillage areas during the critical period for the maize crop (V6) was accompanied by an increase in grain yield from 547.64 kg ha⁻¹ to 3330.60 kg ha⁻¹, respectively. In the ICLS areas, on the other hand, there was no significant difference between the N- and N+ areas (Figure 5).

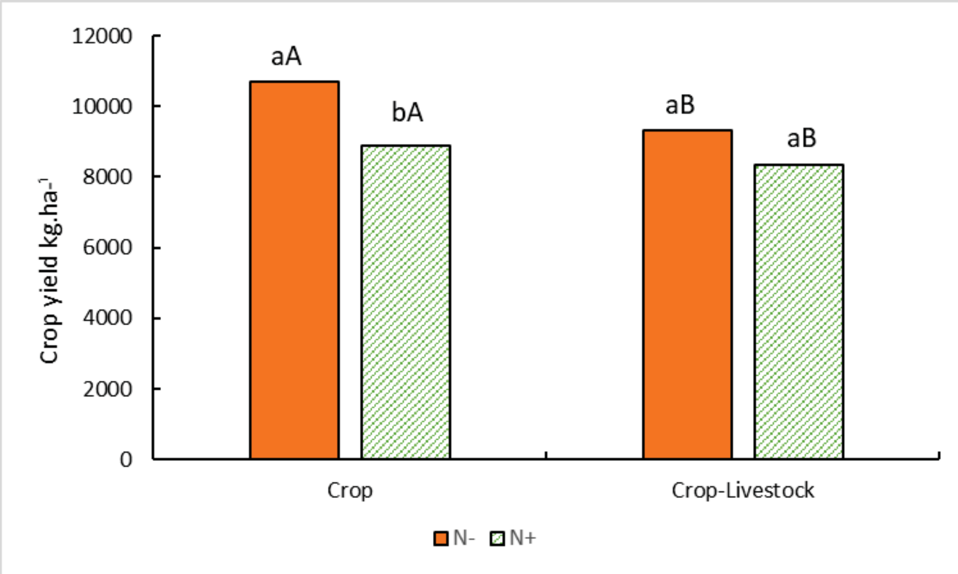
Figure 5 Maize crop yield in the MCS and ICLS and two doses of N. N- (without N fertilization) and N+ (with application of 180 kg N ha^{-1} when the maize had three fully expanded leaves), in a seven-year crop-livestock-forest integration experiment – NITA in Pinhais, Paraná, Brazil, in the 2021-2022 harvest.



*Interpretation: Averages followed by different lowercase letters compare the N fertilization in the systems, and the uppercase letters highlight the differences between the systems in the N fertilization treatments, according to the Tukey test at $p<0.05$.

The yields found at Fazenda Capão Redondo responded to nitrogen fertilization. The areas that received only N during the winter (N-) showed an increase of around 5547 kg ha^{-1} (Figure 6).

Figure 6 Maize crop yield in the Crop and Crop-Livestock systems and two doses of N. N- (without N fertilization) and N+ (with an application of 180 kg N ha^{-1} when the maize had three fully expanded leaves), on a Capão Redondo- Candói farm, Paraná, Brazil, in the 2021-2022 agricultural season.



*Interpretation: Averages followed by different lowercase letters compare the N fertilization in the systems, and the uppercase letters highlight the differences between the systems in the N fertilization treatments, according to the Tukey test at $p<0.05$.

4 DISCUSSIONS

4.1 NNI, NH_4^+ and CMB during winter grass cultivation

Nitrogen fertilization is a key factor in the development of grasses, which have a positive response in terms of yield. However, NNI concepts have been little applied in areas that have adopted ICLS and in subtropical environments, where nitrogen fertilization management tends to be differentiated in terms of doses and times of application.

Our work revealed that the NNI values of the grasses established in the L area at NITA during the winter were higher than those found in the ICLS areas until the 3rd evaluation period (Table 3), despite the fact that the concentration of N decreased as the biomass of the aerial part increased, due to the dilution of the nutrient. The NNI values showed that the plant was deficient during the evaluations, i.e. the 90 kg N ha^{-1} applied was unable to meet the need for the nutrient. In the areas where the grasses were used for winter cover (L), the C:N ratio is higher and, although the N levels were higher in the L areas, the need of the microorganisms, justified by the higher CMB found, to consume this excess C may have led to a temporary immobilization of this N, making it unavailable (Apolinário et al., 2013, Kaschuk et al., 2010).

On the other hand, in the crop-livestock systems (ICLS) areas, results showed that there was a higher consumption of N (NNI > 1) during the 4th evaluation period. This evaluation period was carried out 30 days after the animals were removed from the area to plant maize. During grazing, canopy defoliation is constant and, although part of the N consumed is returned in high concentrations through urine deposition (Soussana and Lemaire, 2014), the plant was constantly growing and had low leaf area, leading to low N dilution and consequently low NNI values (Lemaire et al., 2008). After the grazing period, the increase in NNI was evident, as the plants reached their isometric growth with high N absorption (Lemaire et al., 2008).

As a fundamental part of the N cycle, microbial activity is strongly related to the availability of organic substrates, such as soil organic matter and crop residues, which are sources of carbon for microorganisms (Apolinário et al., 2013, Kaschuk et al., 2010). The increase in CMB found in the NITA farm area indicates a greater capacity for decomposing organic matter and greater metabolic activity by microorganisms, resulting in a higher concentration of nutrients, including ammonium (Table 1).

At the Capão Redondo farm (Table 2), both treatments evaluated had NNI > 1, showing that there was better absorption of the nutrient at this site, probably due to the type of grazing used in the area, which allowed the plant to recover from the defoliation caused by the animals. In addition, the presence of animals can reduce the C:N ratio of the waste, which is due to the temporary immobilization of N by soil microorganisms when they consume excess C and achieve synchrony between the release of nutrients, including ammonium, and plant absorption (Maccari et al., 2021).

4.2 NNI, NH_4^+ and CMB during maize cultivation

The absorption of nutrients, especially N, is regulated by plant growth (Lemaire et al., 2008), but this growth is variable and depends on temperature, water and light availability, genotype and management. In fact, the impact of using ICLS intensifies nutrient cycling and the activity of the soil's microbial biomass, whether due to the management adopted or the presence of animals in these areas (Soussana and Lemaire 2014; Moraes et al., 2019; Sarto et al., 2020; Valani et al., 2020; Franzluebbers, 2018; Franzluebbers, 2020). The presence of grazing animals decouples the C and N cycles by returning the available N in high concentrations, allowing it to be passed on to successor crops (Maccari et al., 2021).

As expected, the concentration of N in the aerial part of the maize decreased with the accumulation of dry biomass only in the L system with the application of N in the NITA (Table 3), corroborating the model presented by Gastal et al., 2015. During the initial stages of plant growth, N concentrations are relatively high, because the plant has less dry mass and requires less nutrients to sustain its initial growth, resulting in higher N levels. As the plant's dry mass increases, there is a process of redistribution of nitrogen (N) from the older vegetative parts to the younger ones, resulting in the process of N dilution (Lemaire et al., 2008; Soussana and Lemaire, 2014).

The presence and availability of NH_4^+ (Table 3) as well as productivity (Figure 4) were dependent on the application of N in the LN- areas. The application of nitrogen fertilizers in agricultural areas promotes the immediate availability of N to plants, and part is immobilized by soil microorganisms for later release. However, we observed no differences between the microbial activity of the soil in the LN- and LN+ areas, indicating that in the NITA farming areas there was competition for the nutrient between the plants and the microorganisms for N.

One of the main advantages of ICLS is the efficiency achieved in nutrient cycling, especially N, due to the presence of animals in the areas (Moraes et al., 2019). During grazing, there is decoupling of C and N, which occurs due to changes in the C:N ratios between the remaining vegetation and the excreta deposited by the animals (Soussana and Lemaire, 2014). This decoupling implies greater availability of nutrients in the system, which can result in increased agricultural production.

In this study, in the ICLS areas, the NNI, NH_4^+ and CMB of the N- areas were constant during the evaluation period (Table 3), demonstrating that nitrogen absorption in these areas was not limited by nitrogen fertilization. These results show that there was no competition between the soil microbial biomass and the plant for the nutrient and corroborate some studies (Assmann et al., 2017, Moraes et al., 2020; Alves et a., 2020; Adetunji et al., 2019) which have demonstrated the effectiveness of grazing as a management strategy to promote plant recovery and growth. Soil microbial biomass is influenced by several factors, such as nutrient availability, soil and climate conditions and intrinsic relationships in the soil-plant-microorganism system (Kaschuk et al., 2010; Anzalone et al., 2020; Lazeris et al., 2021; Ferreira et al., 2021). Although we found differences in NNI in the ICLS N+ areas (Table 3), these were not enough to limit the growth of the maize crop, which responded in productivity (Figure 5) and increased NNI at the end of its cycle.

Although there are numerous studies highlighting the importance of nitrogen fertilization management in agricultural systems (Assmann et al., 2017; Maccari et al., 2020), few highlight the importance of microbial biomass on soil quality and crop productivity. On the Capão Redondo farm, NNI levels were greater than 1 in both systems studied. This means that the addition of nitrogen through fertilizer application compensated for the decrease in availability of this nutrient as NH_4^+ decreased (Table 4).

5 CONCLUSIONS

This study reinforces the importance of nutrient enrichment in the soil's microbial biomass, as we have seen a direct relationship between the resilience of the soil's microbial biomass, NNI and the response to nitrogen fertilization. These factors show a significant interrelationship, directly influencing the competition for nutrients between plants and the soil microbial biomass.

Our results highlight the need to promote appropriate management practices aimed at supplying nutrients to the soil microbial biomass in order to optimize fertilization efficiency and the sustainability of agricultural ecosystems, such as implementing strategies that preserve soil biomass and promote a positive interaction between plants and soil microorganisms.

We emphasize the importance of future research to further explore this complex relationship, with a view to developing more sustainable and efficient agricultural practices.

6 REFERENCES

Adetunji, A.T., Ncube, B., Mulidzi, R., Lewu, F.B. (2020). Management impact and benefit of cover crops on soil quality: A review. Soil Tillage Reach., 204:104717, DOI: 10.1016/j.still.2020.104717

Alves, L.A, Denardin, L.G.O., Martins, A.P., Anghinoni, I., Carvalho, P.C.F., Tiecher, T. Soil acidification and P, K, Ca, and Mg budget as affected by sheep grazing and crop rotation in a long-term integrated crop–livestock system in southern Brazil (2019). Geoderma. 351:197-208. https://doi.org/10.1016/j.geoderma.2019.04.036

Anzalone, R., Vezzani, F.M., Kaschuk, G., Hungria ,, M., Vargas, L., Nogueira, M.A. (2020). Establishing reference values for soil microbial biomass-C in agroecosystems in the Atlantic Forest Biome in Southern Brazil. Ecological IndicartorsIndicators, 117:106586. https://doi: 101016/j.ecolind.2020.106586

APHA - American Public Health Association American Water Works Association, Water Environment Federation. Standard Methods for the Examination of Water and Wastewater. 21th. ed. Washington DC. APHA-WEF, 1468p. 2005

Bernardon, A., Assmann, T.S., Soares, A.B., Franzluebbers A., Maccari M., Bortolli, M.A.(. (2020). Carryover of N-fertilization from maize to pasture in an integrated crop-livestock system. Archives Agronomy Soil Science, 66:1-16. doi: 10.1080/03650340.2020.1749268

A.A. Balbinot Júnior, A. Moraes, M. Veiga, A. Pelissari, J. Dieckow. Performing of winter crops at yield of matter and soil cover on monocropping and intercropping systems Rev. Cien. Agrovet., 3 (2004), pp. 38-42

T.S. Assmann, A.B. Soares, A.L. Assmann, F. Levinski, R. Corrêa Adubação de Sistemas em Integração Lavoura-Pecuária. Proceedings of the 1st Congresso Brasileiro de Sistemas Integrados de Produção Agropecuária; Aug 21–24; Cascavel, Brazil, UTFPR, Pato Branco (2017)

M.F. Cotrufo, D.W. Matthew, M.B. Claudia, D. Karolien, P. Eldor. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form sTable soil organic matter? Glob. Chang. Biol., 19 (2013), pp. 988-995

Costa, N.D.L., Gianluppi, V., Moraes, A.,Carvalho, P.C.F., Magalhães, J.A.(2017). Nitrogen nutrition index of Trachypogon plumosus pastures in Roraima's savannas. Pubvet, 11:825-831. https://doi:10.22256/PUBVET.V11N8.825-831

R. Cavicchioli, W.J. Ripple, K.N. Timmis, F. Azam, L.R. Bakken, M. Baylis, et al. Scientists' warning to humanity: microorganisms and climate change Nat. Rev. Microbiol., 17 (2019), pp. 569-586, doi: 10.1038/s41579-019-0222-5

J.M. Damian, E.S. Matos, B.C. Pedreira, P.C.F. Carvalho, L.M. Premazzi, S. Williams, K. Paustian, C.E.P. Cerri. Predicting soil C changes after pasture intensification and diversification in Brazil, Catena, 202 (2021), pp. 105-238, doi: 10.1016/j.catena.2021.105238

Ferreira, G.W.D., Rau, B.M., Aubrey, D.P.(. (2021). Temporal nitrogen dynamics in intensively managed loblolly pine early stand development. Forest Ecology Management, 48:118890. https://doi: 10.1016/j.foreco.2020.118890

M. Hartmann, B. Frey, J. Mayer, P. Mader, F. Widmer. Distinct soil microbial diversity under long-term organic and conventional farming.ISME J., 9 (2015), pp. 1177-1194, doi:10.1038/ismej.2014.210

Kaschuk, G., Alberton, O., Hungria, M. (2010). Three decades of soil microbial biomass studies in Brazilian ecosystems: Lessons learned about soil quality and indicators for improving sustainability. Soil Biol. Biochem. 42:1-13. doi: https://doi.org/10.1016/j.soilbio.2009.08.020

Franzluebbers, A.(. (2020). Soil-test biological activity with the flush of CO2: Validation of nitrogen prediction for maize production. Agronomy Journal, 112:2188-2204. https://doi: 10.1002/agj2.20094

Franzluebbers, A.(. (2018). Soil-test biological activity with the flush of CO2: III Maize yield responses to applied nitrogen. Soil Science Society of America Journal, 82:708-721.

Lemaire, G., Jeuffroy, M.H., Gastal, F. (2008). Diagnosis tool for plant and crop N status in vegetative stage Theory and practices for crop N management. European Journal of Agronomy, 28:614-624. https://doi: 10.1016/j.eja.2008.01.005

Lazeris, T.S., Souza, J.P., Vezzani, F.M., Lima, C.M., Kaschuk, G. (2021). Carbon and phosphorus biogeochemical cycles in native forest and horticultural areas in the

Metropolitan Region of Curitiba, Brazil. Colloquium Agrariae, 17:1-11. https://doi:10.5747/ca.2021

Lemaire, G., Gastal, F. (1997). N uptake and distribution in plant canopies. In: Lemaire G, (ed.). Diagnosis of the nitrogen status in crops. Heidelberg: Spring-Verlag. 239 p.

Moraes, A., Carvalho, P.C.F., Crusciol, C.A.C., Lang, C.R., Pariz, C.M., Deiss, L., Sulc, M.R. (2019). Integrated Crop-Livestock Systems as a Solution Facing the Destruction of Pampa and Cerrado Biomes in South America by Intensive Monoculture Systems. In: Agroecosystem Diversity: Reconciling Contemporary Agriculture and Environmental Quality. Elsevier: Amsterdam, The Netherlands (2019) p.257–273, https://doi: 10.1016/B978-0-12-811050-8.00016-9

Moraes, A., Carvalho, P.C.F., Anghinoni, I., Lustosa, S.B.C., Costa, S.E.V.G.A., Kunrath, T.R. (2014). Integrated crop–livestock systems in the Brazilian subtropics. European Journal of Agronomy, 57:4–9. https://doi:10.1016/j.eja.2013.10.004

Martins, A.P., Cecagno, A.P. D., Borin, J.B.M., Arnuti, F., Lochmann, S.H., Anghinoni, I., Bissani, C.A., Bayer, C., Carvalho, P.C.F. (2016). Long-, medium- and short-term dynamics of soil acidity in an integrated crop–livestock system under different grazing intensities. Nutr. Cycl. Agroecosys., 104: 67-77. Doi: https://10.1007/s10705-015-9759-5

M. Maccari, T.S. Assmann, A. Bernardon, A.B. Soares, A. Franzluebbers, M. Bortolli, B.B. Bortolli, C.L. Glienke. Relationships between N, P, and K in maize biomass for assessing the carryover effects of winter pasture to maize. Eur. J. Agron. (2021), doi: 10.1016/j.eja.2021.126317

Gastal, F., Lemaire, G., Durand, J.L., Louarn, G. (2015). Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. Crop Physiology, 2:161-206. https://doi: 10.1016/B978-0-12-417104-600008-X

Kruchelski, S., Trautenmüller, J.W., Deiss, L., Trevisan, R., Cubbage, F., Porfírio-da-Silva, V., Moraes, A.(. (2021). Eucalyptus benthamii Maiden et Cambage growth and wood density in integrated crop-livestock systems. Agrofororesty System, 95: 1577–1588. https://doi:10.1007/s10457-021-00672-0

Pauletti, V., Motta, A.C.V. (2019). Manual de adubação e calagem para o estado do Paraná. SBCS - Sociedade Brasileira de Ciência do Solo. Curitiba-PR. 289 p.

Ruthes, B.E.S., Kaschuk, G., de Moraes, A. et al. Soil Microbial Biomass, N Nutrition Index, and Yield of Maize Cultivated Under eucalyptus Shade in Integrated crop-livestock-forestry Systems. Int. J. Plant Prod. 17, 323–335 (2023). https://doi.org/10.1007/s42106-023-00242-7

Santos, H.G., Jacomine, P.K.T., Anjos, L.H.C., Oliveira, V.A., Lumbreras, J.F., Coelho, M.R., Almeida, J.A., Filho, J.C.A., Oliveira, J.B., Cunha, T.J.F. (2018). Sistema brasileiro de classificação de solos. Brasilia, DF: Embrapa (2018). 355p.

L. Stefan, M. Hartmann, N. Engbersen, J. Six, C. Schöb. Positive effects of crop diversity on productivity driven by changes in soil microbial composition. Front. Microbiol., 12 (2021), p. 808, doi:10.3389/fmicb.2021.660749

Sampaio, E.V.S.B., Salcedo, I.H., Maia, L.C. (1986). Limitações no cálculo da biomassa microbiana determinada pelo método da fumigação em solos com adição recente de substrato orgânico (14C). Revista Brasileira de Ciência do Solo. (1986) 10:31-35.

J. Soussana, G. Lemaire. Coupling carbon and nitrogen cycles for environmentally sustainable intensification of grasslands and crop-livestock systems. Agric. Ecosyst. Environ., 190 (2014), pp. 9-17, doi: 10.1016/j.agee.2013.10.012

Sarto, M.V.M., Borges, W.L.B., Sartom J.R.W., Rice, C.W., Rosolem, C.A. (2020). Root and shoot interactions in a tropical integrated crop–livestock–forest system. Agricultural Systems, 181:102796. https: doi://101016/j.agsy2020.102796

Sandini, I. E., Moraes, A., Pelissari, A., Neumann, M., Falbo, M. K., & Novakowiski, J. H. (2011). Efeito residual do nitrogênio na cultura do milho no sistema de produção integração lavoura-pecuária. Ciência rural, 41(8), 1315–1322.

Tedesco, M.J., Gianello, C., Bissani, C.A., Bohnen, H., Volkweiss, S.J. (1995). Análises de solo, plantas e outros materiais. Porto Alegre, Universidade Federal do Rio grande do Sul. 174p.

USDA-United States Department of Agriculture: Soil survey staff Keys to soil taxonomy, USDA-Natural Resources Conservation Service, Washington, DC. (2014). 372p.

Vance, E.D., Brookes. P.C., Jenkinson, D.S. (1987). An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemestry, 9:703-707. https://doi:10.1016/0038-0717(87)90052-6

Valani, G.P., Martíni, A.F., Silva, L.F.S., Bovi, R.C., Cooper. M. (2020). Soil quality assessments in integrated crop–livestock–forest systems: A review. Soil Use and Management, 37:22-36, https://doi:10.1111/sum12667

W. Zhou, T.F. Lv, Y. Chen, A.P. Westby, W.J. Ren .Soil physicochemical and biological properties of paddy-upland rotation: a review. Sci. World J. (2014), doi: 10.1155/2014/856352

Y. Yu, L. Xue, L. Yang. Winter legumes in rice crop rotations reduces nitrogen loss, and improves rice yield and soil nitrogen supply. Agron. Sustain. Dev., 34 (2014), pp. 633-640

B. Zhu, L. Yi, L. Guo, et al. Performance of two winter cover crops and their impacts on soil properties and two subsequent rice crops in Dongting Lake Plain, Hunan, China. Soil Tillage Res., 124 (2012), pp. 95-101

WHITEHEAD, D.C. Nitrogen. In: WHITEHEAD, D.C. (Ed.) Nutrient Elements in Grassland: soil-plant-animal relationships. Wallingford, UK: CAB Internacional, 2000. p. 95-125.

FINAL CONSIDERATIONS

This study examined the interaction between animals, plants and soil microbial biomass in Integrated Crop-Livestock Systems (ICLS) and how this affects nutrient cycling and grain yields. The results highlight the importance of the presence of animals in ICLS, both to improve soil fertility and to facilitate the efficient recycling of nutrients.

In addition to the presence of trees in ICLS, which play a crucial role in climate regulation, pest control and nutrient cycling, the presence of grazing animals improves soil nutrition and fertility by promoting microbial activity and nutrient mineralization. These processes are crucial for preserving soil health and maximizing crop yields.

The interaction between animals, plants and soil microbial biomass fosters a more dynamic and balanced nutrient cycle, resulting in higher soil biological quality and grain productivity. The presence of animals stimulates the activity of the microbial biomass, favoring the efficient transfer of nutrients to subsequent crops.

However, it is essential to implement appropriate management to optimize this interaction. Strategies such as grazing management and the application of nitrogen fertilizers should be considered to ensure the efficient absorption of nutrients by plants and minimize losses. In addition, it is essential to assess the particularities of each production system and region, in order to adapt management according to their needs and maximize efficiency in the use of nutrients.

The importance of the interaction between animals, plants and the soil's microbial biomass in ICLS to promote more sustainable and effective agricultural practices. Understanding these complex interactions can guide the development of appropriate management strategies aimed at efficient nutrient recycling, soil health and crop productivity. Future research should focus on deepening our understanding of these mechanisms and further exploring the potential of ICLS in sustainable agriculture.

GENERAL REFERENCES

Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.D.M., Sparovek, G. (2013). Köppen's climate classification map for Brazil. Meteorologische Zeitschrift.22:6. doi: https://10.1127/0941-2948/2013/0507

Aleixo, A.P., Kaschuk, G., Alberton O. (2014). Soil fungal and bacterial biomass determined by epifluorescence microscopy and mycorrhizal spore density in different sugarcane managements. Ciência Rural, 44:588-594. https://doi:10.1590/S010384782014000400002

Batista E.R., Zanchi C.S., Ferreira D.A., Santiago F.L.A., Pinto F.A., Santos J.V., Paulino H.B., Carneiro M.A.C. (2018). Atributos biológicos do solo em sistema integrado de produção agropecuária. In: Souza E.D., Silva F.D., Assmann T.S., Carneiro M.A.C., Carvalho P.C.F., Paulino H.B. (Eds). Sistemas Integrados de Produção Agropecuária no Brasil. Tubarão, SC. p.71-90.

Bremner, J.M. (1965). Total nitrogen. In: Black, C.A., (Eds). Methods of soil analysis Madison: American Society of Agronomy. Madison, W.I. p.1149-1178

Bieluczyk, W., Piccolo, M.C. Pereira, M.G., Moraes, M.T., Amin, S.A., Bernardi,A.C.C., Pezzopane, J.R.M., Oliveira, P.P.A, Moreira, M.Z., Camargo, P.B., Dias, T.S., Batista, I., Cherubin, M.R. (2020). Integrated farming systems influence soil organic matter dynamics in southeastern Brazil. Geoderma, v.371. https://doi:10.1016/j.geoderma.2020.114368

Bell, J.M., Robinson, C.A., Schwartz, R.C. (2006). Changes in soil properties and enzymatic activities following manure applications to a Rangeland. Rangeland Ecol. Manage., 56: 314-320. doi: https://doi.org/10.2111/05-172R1.1

Costa, N.D.L., Gianluppi, V., Moraes, A.,Carvalho, P.C.F., Magalhães, J.A.(2017). Nitrogen nutrition index of Trachypogon plumosus pastures in Roraima's savannas. Pubvet, 11:825-831. https://doi:10.22256/PUBVET.V11N8.825-831

CONAB (2020). Cost of Agricultural Production. Available online at: http://www.conab.gov.br/conab/Main.php?MagID=3&MagNo=39 (accessed November 30, 2022).

Carrer, M.J., Maia, A.G., Vinholis, M.M.B., Filho, H.M.S. (2020). Assessing the effectiveness of rural credit policy on the adoption of integrated crop-livestock systems in Brazil, Land Use Policy 92:104468. https://doi:10.1016/j.landusepol.2020.104468

Deiss, L., Kleina, G.B., Moraes, A., Franzluebbers, A.J., Motta, A.C.V., Dieckow, J., Sandini, I.E., Anghinoni, I., Carvalho, P.C.F. (2019). Soil chemical properties under no-tillage as affected by agricultural trophic complexity. European Journal Soil Science.171:1090-¹105. https://doi:10.1111/ejss.12869

Derpsch, R., and Friedrich, T. (2009). “Development and current status of no-till adoption in the world,” in Proceedings on CD 18th Triennial Conference of the International Soil Tillage Research Organization (Izmir: ISTRO), 15–19.

Du, L., Li, Q,. Li, L., Wu, Y., Zhou, F., Liu, B., Zhao, B., Li, X., Liu, Q., Kong, F., Yuan, J. (2020) Construction of a critical nitrogen dilution curve for maize in Southwest China. Scientific Reports, 10:13084. https://doi:10.1038/s41598-020-70065-3

Embrapa (2020). Adoção de sistemas de integração lavoura-pecuária-floresta (IICLSF) em São Paulo. Boletim de Pesquisa e Desenvolvimento. ISSN: 1980-6841.

56p.

FAOSTAT (2023) Food and Agriculture Organization of the Nations (FAO). FAOSTAT Database. Crops and livestock products. https://www.fao.org/faostat/en/#data/QCL

Faissal, A., Ouazzani, N., Parrado, J.R., Dary, M., Manyani, H., Morgado, B.R., Barragán, M.D., Mandi, L. (2017). Impact of fertilization by natural manure on the microbial quality of soil: Molecular approach. Saudi Journal of Biological Science, 24:1437-1443. https://10.1016/j.sjbs.2017.01.005

Feije, F., Anger, V. (1972). Spot test in inorganic analysis. Analytica Chimica Acta. 149: 363-367

Ferreira, G.W.D., Rau, B.M., Aubrey, D.P. (2021). Temporal nitrogen dynamics in intensively managed loblolly pine early stand development. Forest Ecology Management, 48:118890. https://doi: 10.1016/j.foreco.2020.118890

Franzluebbers, A. (2020). Soil-test biological activity with the flush of CO2: Validation of nitrogen prediction for maize production. Agronomy Journal, 112:2188-2204. https://doi: 10.1002/agj2.20094

Franzluebbers, A. (2018). Soil-test biological activity with the flush of CO2: III Maize yield responses to applied nitrogen. Soil Science Society of America Journal, 82:708721. Htts://doi:10.2136/sssaj2018.01.0029

Fu, Y., Li. H.Y., Yu, J., Liu, H., Cao, Z., Manukovsky, N.S., Liu, H. (2017). Interaction effects of light intensity and nitrogen concentration on growth, photosynthetic characteristics and quality of lettuce (Lactuca sativa L. var. youmaicai). Scientia Horticulturae, 214:51-57. https://doi:10.1016/j.scienta.2016.11.020.

Gastal, F., Lemaire, G., Durand, J.L., Louarn, G. (2015). Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. Crop Physiology, 2:161206. https://doi: 10.1016/B978-0-¹2-417104-600008-X

Geremia, E.V., Crestani, S., Mascheroni, J.D.C., Carnevalli, R.A., Mourão, G.B., Silva, S.C. (2018). Sward structure and herbage intake of Brachiaria brizantha cv Piatã in a crop-livestock-forestry integration area. Livestock Science, 212: 83-92. https://doi:10.1016/j.livsci.2018.03.020

Gonzalez-Quiñones, V., Stockdale, E.A., Banning, N.C., Hoyle, F.C., Sawada, Y., Wherrett, A.D., Murphy, D.V. (2011). Soil microbial biomass: Interpretation and consideration for soil monitoring. Soil Research, 49: 287-304.

Gamboa, C.H., Vezzani, F.M., Kaschuk, G., Favaretto, N., Cobos, J.Y.G., Costa, G.A. (2020). Soil-Root Dynamics in Maize-Beans-Eggplant Intercropping System under Organic Management in a Subtropical Region. Journal Soil Science and Plant Nutrition, 20:1480–1490. https://doi:10.1007/s42729-020-00227-9

Glatzle, S., Stuerz, S., Giese, M., Pereira, M., Almeida, R.G., Bungenstab, D.J., Macedo, M.C.M., Asch, F. (2021). Seasonal dynamics of soil moisture in an integrated-crop-livestock-forestry system in Central-West Brazil. Agriculture, 11:245. https://doi:10.3390/agriculture11030245

Haney, R.L., Franzluebbers, A.J., Porter, E.B., Hons, F.M., Zuberer, D.A. (2004). Soil carbon and nitrogen mineralization: Influence of drying temperature. Soil Science Society of America Journal, 68:489-492. doi: 10.2136/sssaj2004.4890

Himmelstein, J., Ares, A., Gallagher, D., and Myers, J. (2017). A meta-analysis of intercropping in Africa: impacts on crop yield, farmer income, and integrated pest management effects. Int. J. Agric. Sustain. 15, 1–10. doi: 10.1080/14735903.2016.1242332

Kaschuk, G., Alberton, O., Hungria, M. (2010). Three decades of soil microbial biomass studies in Brazilian ecosystems: Lessons learned about soil quality and indicators for improving sustainability. Soil Biol. Biochem. 42:1-13. https://doi.org/10.1016/j.soilbio.2009.08.020

Kaschuk, G., Hungria, M. (2017). Diversity and importance of diazotrophic bacteria to agricultural sustainability in the Tropics. In: de Azevedo J, Quecine M. (eds) Diversity and benefits of microorganisms from the tropics. p.269-298. https://doi:10.1007/978-3-319-55804-2_12

Kaschuk, G., Galli-Terasawa, L.V., Albernas, K.K., Ripka, L.P., Pansolin, I.C., Matos, C.L., Pauletti, V., Kava, V. (2022). Inoculation of plant growth promoting Bacillus spp. in N-fertilized maize crop in soils with high organic matter content in South Brazil. Journal Agriculture Science, 14: 43-52 https://doi.org/10.5539/jas.v14n7p43

Kruchelski, S., Trautenmüller, J.W., Deiss, L., Trevisan, R., Cubbage, F., Porfírio-daSilva, V., Moraes, A. (2021). Eucalyptus benthamii Maiden et Cambage growth and wood density in integrated crop-livestock systems. Agrofororesty System, 95: 1577– 1588. https://doi:10.1007/s10457-021-00672-0

Karvatte, N., Klosowski, E.S., Almeida, R.G., Mesquita, E.E., Oliveira, C.C., Alves, F.V. (2016). Shading effect on microclimate and thermal comfort indexes in integrated crop-livestock-forest systems in the Brazilian Midwest. International Journal of Biometeorology, 60:1933–1941 https://doi: 10.1007/s00484-016-1180-5

Moraes, A., Carvalho, P.C.F., Crusciol, C.A.C., Lang, C.R., Pariz, C.M., Deiss, L., Sulc, M.R. (2019). Integrated Crop-Livestock Systems as a Solution Facing the Destruction of Pampa and Cerrado Biomes in South America by Intensive Monoculture Systems. In: Agroecosystem Diversity: Reconciling Contemporary Agriculture and Environmental Quality. Elsevier: Amsterdam, The Netherlands (2019) p.257–273, https://doi: 10.1016/B978-0-12-811050-8.00016-9

Moraes, A., Carvalho, P.C.F., Anghinoni, I., Lustosa, S.B.C., Costa, S.E.V.G.A., Kunrath, T.R. (2014). Integrated crop–livestock systems in the Brazilian subtropics. European Journal of Agronomy, 57:4–9. https://doi:10.1016/j.eja.2013.10.004

Lu, D., Sun, X., Wang, X., Yan, F., Lu, W. (2013). Effect of shading during grain filling on the physicochemical properties of fresh waxy maize. Journal of Integrative Agriculture, 12:1560-¹567. https://doi:10.1016/S2095-3119(13)60560-2.

Liang, X.G., Gao, Z., Shen, .S, Paul, M.J., Zhang, L., Zhao, X., Lin, S., Wu, G., Chen, X.M., Zhou, S.L. (2020). Differential ear growth of two maize varieties to shading in the field environment: Effects on whole plant carbon allocation and sugar starvation response. Journal of Plant Physiology, 251:153-194. https://doi:10.1016/j.jplCLSh.2020.153194

Lopes, A.A.C., Sousa, D.M.G., Reis Junior, F.B., Figueiredo, C.C., Malaquias, J.V., Souza, L.M., Mendes, I.C. (2018). Temporal variation and critical limits of microbial indicators in oxisols in the Cerrado, Brazil. Geoderma, 12:72-82. https://doi:10.1016/j.geodrs.2018.01.003

Lazeris, T.S., Souza, J.P., Vezzani, F.M., Lima, C.M., Kaschuk, G. (2021). Carbon and phosphorus biogeochemical cycles in native forest and horticultural areas in the Metropolitan Region of Curitiba, Brazil. Colloquium Agrariae, 17:1-11. https://doi:10.5747/ca.2021

Lemaire, G., Jeuffroy, M.H., Gastal, F. (2008). Diagnosis tool for plant and crop N status in vegetative stage Theory and practices for crop N management. European Journal of Agronomy, 28:614-624. https://doi: 10.1016/j.eja.2008.01.005

Lemaire, G., Franzluebbers, A., Carvalho, P.C.F., Dedieu, B. (2014). Integrated crop–livestock systems: Strategies to achieve synergy between agricultural production and environmental quality. Agriculture Ecosystem Environment, 190:4-8. https://doi:10.1016/j.agee.2013.08.009

Lemaire, G., Gastal, F. (1997). N uptake and distribution in plant canopies. In:Lemaire G, (ed.). Diagnosis of the nitrogen status in crops. Heidelberg: Spring-Verlag. 239 p.

Mu, X., Chen, Y. (2021). The physiological response of photosynthesis to nitrogen deficiency. Plant Physiology Biochemstry,158:76-82. https://doi: 10.1016/j.plaphy.2020.11.019

Pacín, F., Oesterheld, M. (2014). In-farm diversity stabilizes return on capital in Argentine agro-ecosystems. Agriculture Systems, 124: 51–59. https://doi:10.1016/j.agsy.2013.10. 008.

Paes, L.S.D.O.P., Lima, M.R., Kaschuk, G., Vezzani, F.M. (2018). Root development as an indicator of soil sustainability of cropping systems in the Atlantic Forest Biome. Cultivos tropicales, 39: 7-14.

Perosa, B., Newto, P., Carrer, M.J. (2021). Access to information affects the adoption of integrated systems by farmers in Brazil. Land Use Policy, https://doi:10.1016/j.landusepol.2021.105459

Peterson, C.A., Deiss, L., Gaudin, A.C.M. (2020). Commercial integrated croplivestock systems achieve comparable crop yields to specialized production systems: A meta-analysis. Plos One 15:0231840. https://doi:10.1371/journal.pone.0231840

Pauletti, V., Motta, A.C.V. (2019). Manual de adubação e calagem para o estado do Paraná. SBCS - Sociedade Brasileira de Ciência do Solo. Curitiba-PR. 289 p.

Pariz, C. M., Costa, C., Crusciol, C. A. C., Castilhos, A. M., Meirelles, P. R. L., Roça, R. O., et al. (2017). Lamb production responses to grass grazing in a companion crop system with maize silage and oversowing of yellow oat in a tropical region. Agriculture Systems 151, 1–11. doi: 10.1016/j.agsy.2016.11.004

Pezzopane, J., Bonani, W., Bosi, C., Fernandes, E.R., Campos, B.A., Oliveira, P., Pedroso, F.A. (2020). Reducing competition in a crop–livestock–forest integrated system by thNNIing eucalyptus trees. Experimental Agriculture, 56:574-586. https://doi:10.1017/S0014479720000162

Pontes, L.S., Tullio, G.F., Martins, A.S., Moletta, J.L., Silva, V.P. (2018). Maize yield for silage and grains in different integrated crop-livestock systems. Revista Ciência Agronômica, 49:315-323. https://doi: 10.5935/1806-669020180036

Pott, L.P., Amando, T.J.C., Schwalbert, R.A., Gebert, F.H., Reimche, G.B., Pes, L.S., Ciampitti, I.A. (2021). Effect of hairy vetch cover crop on maize nitrogen supply and productivity at varying yield environments in Southern Brazil. Science of The Total Environment, 759:144313. https://doi: 10.1016/j.scitotenv.2020.144313

Pico, L.B.O., Vyn, T. (2021). Dry matter gains in maize kernels are dependent on their nitrogen accumulation rates and duration during grain filling. Plants-Basel, 15:10(6):1222. https://doi: 10.3390/plants10061222

Rufino, M.C., Gachene, C.K.K., Rodrigue, V.C.D., Hawkins, J., Onyango, A.A., Sanogo, O.M., Wanyama, I., Yesuf, G., Pelster, D.E. (2021). Sustainable development of crop-livestock farms in África. Frontiers of Agricultural Science and Engineering, 8:175–181. https://doi: 10.15302/J-FASE-2020362

Raij, B.V., Cantarella, H., Quaggio, J.A., Furlani, A.M.C. (1997). Recomendações de adubação e calagem para o Estado de São Paulo. Campinas, SP. Instituto Agronômico/ Fundação IAC. 285p

Reznick. J.P.K., Barth, G., Kaschuk, G., Pauletti, V. (2021). Nitrogen and cultivars as field strategies to improve the nutritional status of wheat grain and flour. Journal of Cereal Science, 102:103290. https://doi: 10.1016/j.jcs.2021.103290

Sekaran, U., Lai, L., Ussiri, D.A., Kumar, S., Clay, S. (2021). Role of integrated croplivestock systems in improving agriculture production and addressing food security–A review. Journal of Agriculture and Food Research, 5:100190. https://doi:

10.1016/j.jafr.2021.100190

Sarto, M.V.M., Borges, W.L.B., Sartom J.R.W., Rice, C.W., Rosolem, C.A. (2020). Root and shoot interactions in a tropical integrated crop–livestock–forest system. Agricultural Systems, 181:102796. https: doi://101016/j.agsy2020.102796

Spohn, M., Chodak, M. (2015). Microbial respiration per unit biomass increases with carbon-to-nutrient ratios in forest soils. Soil Biololgy and Biochemistry, 81:128-133. https://doi:2015EGUGA..17.3838S

Santos, H.G., Jacomine, P.K.T., Anjos, L.H.C., Oliveira, V.A., Lumbreras, J.F., Coelho, M.R., Almeida, J.A., Filho, J.C.A., Oliveira, J.B., Cunha, T.J.F. (2018). Sistema brasileiro de classificação de solos. Brasilia, DF: Embrapa (2018). 355p.

Schroeder, J., Kammann, L., Helfrich, M., Tebbe, C.C., Poeplau, C. (2021). Impact of common sample pre-treatments on key soil microbial properties. Soil Biology and Biochemestry, 160:108321. https://doi: 10.1016/j.soilbio.2021.108321

Souza-Jr, C.M., Shimbo, J.Z., Rosa, M.R., Parente, L.L., Alencar, A., Rudorff, B.F.T., Hasenack, H., Matsumoto, M., Ferreira, L.G., Souza-Filho, P.W.M., Oliveira, S.W., Rocha, W.F., Fonseca, A.V., Marques, C.B., Diniz, C.G., Costa, D., Monteiro, D., Rosa, E.R., Vélez-Martin, E., Weber, E.J., Lenti, F.E.B., Paternost, F.F., Pareyn, F.G.C., Siqueira, J.V., Viera, J.L., Neto, L.C.F., Saraiva, M.M., Sales, M.H., Salgado, M.P.G., Vasconcelos, R., Galano, S., Mesquita, V.V., Azevedo, T. (2020). Reconstructing three decades of land use and land cover changes in Brazilian biomes with Landsat. Archive and Earth Engine. Remote Sensing, 12:2735, https://doi:10.3390/rs12172735

Sampaio, E.V.S.B., Salcedo, I.H., Maia, L.C. (1986). Limitações no cálculo da biomassa microbiana determinada pelo método da fumigação em solos com adição recente de substrato orgânico (14C). Revista Brasileira de Ciência do Solo. (1986) 10:31-35.

Santos, M.V., da Fonseca, D.M., Silva, L.D., Souza, W.F., Oliveira, T.S., Ferreira, L., Paciullo, D.S.C. (2020). Integrated crop–forage–forestry for sustainable agricultural systems: productive performance. Agroforestry Systems, 94:417–427. https://doi:10.1007/s10457-019-00404-5

Tedesco, M.J., Gianello, C., Bissani, C.A., Bohnen, H., Volkweiss, S.J. (1995). Análises de solo, plantas e outros materiais. Porto Alegre, Universidade Federal do Rio grande do Sul. 174p.

Tedesco, M.J., Gianello, C., Anghinoni, I., Bissani, C.A., Camargo, F.A.O., Wiethölter, S. (2004). Manual de adubação e de calagem para os Estados do Rio Grande do Sul e de Santa Catarina. Sociedade Brasileira de Ciência do Solo Comissão de Química e Fertilidade do Solo. Porto Alegre, RS. 376 p.

R Core Team. R: A language and environment for statistical computing. (2022). Vienna, Austria, R Foundation for Statistical Computing. Versão 4.0.2.

USDA-United States Department of Agriculture: Soil survey staff Keys to soil taxonomy, USDA-Natural Resources Conservation Service, Washington, DC. (2014). 372p.

Valani, G.P., Martíni, A.F., Silva, L.F.S., Bovi, R.C., Cooper. M. (2020). Soil quality assessments in integrated crop–livestock–forest systems: A review. Soil Use and Management, 37:22-36, https://doi:10.1111/sum12667

Vance, E.D., Brookes. P.C., Jenkinson, D.S. (1987). An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemestry, 9:703-707. https://doi:10.1016/0038-0717(87)90052-6

Van Keulen, H., Schiere, H. (2004). Crop–livestock systems: old wine in new bottles? In: New directions for a diverse planet. Brisbane, Australia. 12p.

Zhang, T., Chen, H.Y.H., Ruan, H. (2018). Global negative effects of nitrogen deposition on soil microbe. The ISME Jounral. 12:1817–1825. https://doi:10.1038/s41396-018-0096-y

Wei, H., Zhu, Y., Qiu, S., Han, C., Hu, L., Xu, D., Zhou, N., Xing, Z., Hu, Y., Cui, P., Dai, Q., Zhang, H. (2018). Combined effect of shading time and nitrogen level on grain filling and grain quality in japonica super rice. Journal of Integrative Agriculture, 7: 2405-2417. https://doi:10.1016/S2095-3119(18)62025-83

Bernardon, A., Assmann, T.S., Soares, A.B., Franzluebbers A., Maccari M., Bortolli, M.A. (2020). Carryover of N-fertilization from maize to pasture in an integrated croplivestock system. Archives Agronomy Soil Science, 66:1-¹6. doi:10.1080/03650340.2020.1749268

Adetunji, A.T., Ncube, B., Mulidzi, R., Lewu, F.B. (2020). Management impact and benefit of cover crops on soil quality: A review. Soil Tillage Reach., 204:104717, DOI: 10.1016/j.still.2020.104717

Alves, L.A, Denardin, L.G.O., Martins, A.P., Anghinoni, I., Carvalho, P.C.F., Tiecher, T. Soil acidification and P, K, Ca, and Mg budget as affected by sheep grazing and crop rotation in a long-term integrated crop–livestock system in southern Brazil (2019). Geoderma. 351:197-208. https://doi.org/10.1016/j.geoderma.2019.04.036

Anzalone, R., Vezzani, F.M., Kaschuk, G., Hungria ,, M., Vargas, L., Nogueira, M.A. (2020). Establishing reference values for soil microbial biomass-C in agroecosystems in the Atlantic Forest Biome in Southern Brazil. Ecological IndicartorsIndicators, 117:106586. https://doi: 101016/j.ecolind.2020.106586

APHA - American Public Health Association American Water Works Association, Water Environment Federation. Standard Methods for the Examination of Water and Wastewater. 21th. ed. Washington DC. APHA-WEF, 1468p. 2005

Bernardon, A., Assmann, T.S., Soares, A.B., Franzluebbers A., Maccari M., Bortolli, M.A. (2020). Carryover of N-fertilization from maize to pasture in an integrated crop-livestock system. Archives Agronomy Soil Science, 66:1-16. doi: 10.1080/03650340.2020.1749268

A.A. Balbinot Júnior, A. Moraes, M. Veiga, A. Pelissari, J. Dieckow. Performing of winter crops at yield of matter and soil cover on monocropping and intercropping systems Rev. Cien. Agrovet., 3 (2004), pp. 38-42

T.S. Assmann, A.B. Soares, A.L. Assmann, F. Levinski, R. Corrêa Adubação de Sistemas em Integração Lavoura-Pecuária. Proceedings of the 1st Congresso

Brasileiro de Sistemas Integrados de Produção Agropecuária; Aug 21–24; Cascavel, Brazil, UTFPR, Pato Branco (2017)

M.F. Cotrufo, D.W. Matthew, M.B. Claudia, D. Karolien, P. Eldor. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form sTable soil organic matter? Glob. Chang. Biol., 19 (2013), pp. 988-995

R. Cavicchioli, W.J. Ripple, K.N. Timmis, F. Azam, L.R. Bakken, M. Baylis, et al. Scientists' warning to humanity: microorganisms and climate change Nat. Rev. Microbiol., 17 (2019), pp. 569-586, doi: 10.1038/s41579-019-0222-5

J.M. Damian, E.S. Matos, B.C. Pedreira, P.C.F. Carvalho, L.M. Premazzi, S. Williams, K. Paustian, C.E.P. Cerri. Predicting soil C changes after pasture intensification and diversification in Brazil, Catena, 202 (2021), pp. 105-238, doi: 10.1016/j.catena.2021.105238

Ferreira, G.W.D., Rau, B.M., Aubrey, D.P. (2021). Temporal nitrogen dynamics in intensively managed loblolly pine early stand development. Forest Ecology Management, 48:118890. https://doi: 10.1016/j.foreco.2020.118890

M. Hartmann, B. Frey, J. Mayer, P. Mader, F. Widmer. Distinct soil microbial diversity under long-term organic and conventional farming.ISME J., 9 (2015), pp. 1177-1194, doi:10.1038/ismej.2014.210

Kaschuk, G., Alberton, O., Hungria, M. (2010). Three decades of soil microbial biomass studies in Brazilian ecosystems: Lessons learned about soil quality and indicators for improving sustainability. Soil Biol. Biochem. 42:1-13. doi: https://doi.org/10.1016/j.soilbio.2009.08.020

Franzluebbers, A. (2020). Soil-test biological activity with the flush of CO2: Validation of nitrogen prediction for maize production. Agronomy Journal, 112:21882204. https://doi: 10.1002/agj2.20094

Franzluebbers, A. (2018). Soil-test biological activity with the flush of CO2: III Maize yield responses to applied nitrogen. Soil Science Society of America Journal, 82:708721.

Lazeris, T.S., Souza, J.P., Vezzani, F.M., Lima, C.M., Kaschuk, G. (2021). Carbon and phosphorus biogeochemical cycles in native forest and horticultural areas in the

Metropolitan Region of Curitiba, Brazil. Colloquium Agrariae, 17:1-11. https://doi:10.5747/ca.2021

Moraes, A., Carvalho, P.C.F., Crusciol, C.A.C., Lang, C.R., Pariz, C.M., Deiss, L., Sulc, M.R. (2019). Integrated Crop-Livestock Systems as a Solution Facing the Destruction of Pampa and Cerrado Biomes in South America by Intensive Monoculture Systems. In: Agroecosystem Diversity: Reconciling Contemporary Agriculture and Environmental Quality. Elsevier: Amsterdam, The Netherlands (2019) p.257–273, https://doi: 10.1016/B978-0-12-811050-8.00016-9

Martins, A.P., Cecagno, A.P. D., Borin, J.B.M., Arnuti, F., Lochmann, S.H., Anghinoni, I., Bissani, C.A., Bayer, C., Carvalho, P.C.F. (2016). Long-, medium- and short-term dynamics of soil acidity in an integrated crop–livestock system under different grazing intensities. Nutr. Cycl. Agroecosys., 104: 67-77. Doi: https://10.1007/s10705-0159759-5

M. Maccari, T.S. Assmann, A. Bernardon, A.B. Soares, A. Franzluebbers, M. Bortolli, B.B. Bortolli, C.L. Glienke. Relationships between N, P, and K in maize biomass for assessing the carryover effects of winter pasture to maize. Eur. J. Agron. (2021), doi:

10.1016/j.eja.2021.126317

Gastal, F., Lemaire, G., Durand, J.L., Louarn, G. (2015). Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. Crop Physiology, 2:161206. https://doi: 10.1016/B978-0-12-417104-600008-X

Kruchelski, S., Trautenmüller, J.W., Deiss, L., Trevisan, R., Cubbage, F., Porfírio-daSilva, V., Moraes, A.(. (2021). Eucalyptus benthamii Maiden et Cambage growth and wood density in integrated crop-livestock systems. Agrofororesty System, 95: 1577– 1588. https://doi:10.1007/s10457-021-00672-0

Ruthes, B.E.S., Kaschuk, G., de Moraes, A. et al. Soil Microbial Biomass, N Nutrition Index, and Yield of Maize Cultivated Under eucalyptus Shade in Integrated croplivestock-forestry Systems. Int. J. Plant Prod. 17, 323–335 (2023). https://doi.org/10.1007/s42106-023-00242-7

L. Stefan, M. Hartmann, N. Engbersen, J. Six, C. Schöb. Positive effects of crop diversity on productivity driven by changes in soil microbial composition. Front. Microbiol., 12 (2021), p. 808, doi:10.3389/fmicb.2021.660749

J. Soussana, G. Lemaire. Coupling carbon and nitrogen cycles for environmentally sustainable intensification of grasslands and crop-livestock systems. Agric. Ecosyst. Environ., 190 (2014), pp. 9-17, doi: 10.1016/j.agee.2013.10.012

Sandini, I. E., Moraes, A., Pelissari, A., Neumann, M., Falbo, M. K., & Novakowiski, J. H. (2011). Efeito residual do nitrogênio na cultura do milho no sistema de produção integração lavoura-pecuária. Ciência rural, 41(8), 1315–1322.

W. Zhou, T.F. Lv, Y. Chen, A.P. Westby, W.J. Ren .Soil physicochemical and biological properties of paddy-upland rotation: a review. Sci. World J. (2014), doi:

10.1155/2014/856352

Y. Yu, L. Xue, L. Yang. Winter legumes in rice crop rotations reduces nitrogen loss, and improves rice yield and soil nitrogen supply. Agron. Sustain. Dev., 34 (2014), pp. 633-640

B. Zhu, L. Yi, L. Guo, et al. Performance of two winter cover crops and their impacts on soil properties and two subsequent rice crops in Dongting Lake Plain, Hunan, China. Soil Tillage Res., 124 (2012), pp. 95-101

WHITEHEAD, D.C. Nitrogen. In: WHITEHEAD, D.C. (Ed.) Nutrient Elements in Grassland: soil-plant-animal relationships. Wallingford, UK: CAB Internacional, 2000. p. 95-125.