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NITROGEN NUTRITION OF ITALIAN RYEGRASS UNDER LEVELS OF
DIVERSIFICATION IN RICE-BASED INTEGRATED CROP-LIVESTOCK SYSTEMS

Isabella Armiliato Segabinazzi

Porto Alegre
Maio de 2021

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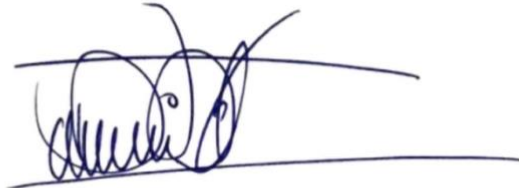
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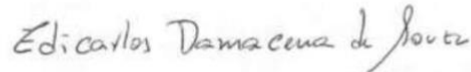
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‘A thin layer of soil covering the surface of the earth is the major interface between agriculture and the environment, and represents the difference between survival and extinction for most land based life’.

J.W. Doran

‘There are only two ways to live your life. One is as though nothing is a miracle. The other is as though everything is a miracle’.

Albert Einstein

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ABSTRACT

This study aimed to assess the nitrogen (N) status of Italian ryegrass (*Lolium multiflorum* Lam.) using as diagnostic tool the Nitrogen Nutrition Index (NNI) in different diversified rice-based Integrated Crop-Livestock Systems (ICLS) in Southern Brazil. Treatments consisted in four ICLS arrangements and three N levels (4 × 3 factorial arrangement), arranged in completely randomized block design with split-plot and three replications. The ICLS arrangements consist in different flooded rice-based cropping systems: 1) Italian ryegrass following a rice monocropping (*Oryza sativa* L.); 2) Italian ryegrass following a rotation of rice and soybean (*Glycine max* (L.) Merr); 3) Italian ryegrass and persian clover (*Trifolium resupinatum* L.) mixed following a rotation of rice, sudan grass (*Sorghum × drummondii* (Steud.) Millsp. & Chas), soybean and corn (*Zea mays* L.) and; 4) Italian ryegrass, persian clover and birdsfoot trefoil (*Lotus corniculatus* L.) mixed following a 3-year rice succession field. The three N levels applied on forage plants were: a) no N application (N0), b) 150 kg ha⁻¹ (N150) and c) 300 kg ha⁻¹ (N300). The increase in diversification of spatio-temporal cultivated plant in ICLS promotes a greater NNI, the S3 and S4 showed the higher NNI. The INN approached the Nc at the N150, but did not reach 1 on average (0.94), while at N300 the points exceeded at all systems (1.14), excepts for S5, and at N0, all systems showed N deficiency. Therefore this work shows that under these field conditions, there is an indication the N levels corresponding to the Nc to Italian ryegrass is between 150 and 300 kg⁻¹ N ha⁻¹.

Keywords: cropping systems, diversification, biological cycling, biogeochemical

NUTRIÇÃO NITROGENADA DE AZEVÉM ITALIANO SOB NÍVEIS DE DIVERSIFICAÇÃO EM SISTEMAS INTEGRADOS DE PRODUÇÃO AGROPECUÁRIA BASEADOS EM CULTIVO DE ARROZ

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RESUMO

Este estudo teve como objetivo avaliar o status de nitrogênio (N) do azevém (*Lolium multiflorum* Lam.) utilizando como ferramenta de diagnóstico o Índice de Nutrição de Nitrogênio (INN) em diferentes Sistemas Integrados de Produção Agropecuária (SIPA) diversificados à base de arroz no sul do Brasil. Os tratamentos consistiram em quatro arranjos de SIPA e três níveis de N (arranjo fatorial 4 × 3), dispostos em delineamento de blocos inteiramente casualizados em split-plot e com três repetições. Os arranjos de SIPA consistem em diferentes sistemas de cultivo à base de arroz irrigado: 1) Azevém após uma monocultura de arroz (*Oryza sativa* L.); 2) Azevém após rotação de arroz e soja (*Glycine max* (L.) Merr); 3) Azevém italiano e trevo persa (*Trifolium resupinatum* L.) misturados após uma rotação de arroz, grama sudão (*Sorghum × drummondii* (Steud.) Millsp. & Chas), soja e milho (*Zea mays* L.) e; 4) Azevém italiano, trevo persa e cornichão (*Lotus corniculatus* L.) misturados após um campo de sucessão de arroz de 3 anos. As três doses de N aplicadas nas plantas forrageiras foram: a) sem aplicação de N (N0), b) 150 kg ha⁻¹ (N150) e c) 300 kg ha⁻¹ (N300). O aumento da diversificação espaço-temporal de plantas cultivadas em SIPA promovem um maior INN, o S3 e S4 apresentaram o maior INN. O INN aproximou-se do N crítico (Nc) no N150, mas não atingiu na média (0.94), enquanto no N300 os pontos superaram o Nc em todos os sistemas (1.14), exceto no S5, e no N0 todos os sistemas apresentaram deficiência de N. Portanto, este trabalho mostra que nessas condições de campo, há uma indicação de que os níveis de N correspondentes ao Nc para o azevém italiano estão entre 150 e 300 kg⁻¹ N ha⁻¹.

Palavras-chave: sistemas de cultivo, diversificação, ciclo biológico, biogeoquímico

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LIST OF ABBREVIATIONS

C: Carbon

C3: Temperate grasses

CEC: Cation-exchange capacity

C:N: Carbon:nitrogen ratio

DM: Dry matter

ha: Hectare

LAI: Leaf area index

ICLS: Integrated Crop-Livestock Systems

M: Million

N: Nitrogen

Na: Current nitrogen content of the crop

Nc: Critical nitrogen content of the crop

NNI: Nitrogen Nutrition Index

NT: No-tillage

SI: Sustainable intensification

t: Ton

LIST OF SYMBOLS

a: Nitrogen concentration when $W = 1 \text{ ton ha}^{-1}$

-b: Dilution coefficient

W: Biomass accumulated in MS ha^{-1} ton in a given time

Cfb: Subtropical humid with mild summers

%: Percentage

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CHAPTER I

1. INTRODUCTION

The world's population is expected to reach 10 billion by 2050, increasing agricultural demand in a modest scenario of economic growth that drives a food transition towards greater consumption of meat, fruits and vegetables. Furthermore, the pressure for natural resources for food production would require structural changes, mainly in cereal production (FAO, 2017).

The transition from diversified and complex to specialized and simple cropping systems resulted from the huge demand for food generated by the population expansion at the end of the 20th century and from a scenario of political and economic support for agricultural production. Agriculture followed the process of specialization, based on input technology and significantly multiplying the capacity to produce food per unit of cultivated area (Anghinoni et al., 2013).

A classic example of specialized system is flooded rice on paddy fields in Rio Grande do Sul (RS). The conventional system of flooded rice cultivation occupies 1 million (M) hectare (ha) annually and is historically characterized by monocropping with winter fallow period, strongly linked to practices of intense and frequent soil tillage (SOSBAI, 2018).

The frequent soil tillage promotes soil disturbance, considered the main cause of soil degradation worldwide (Lal, 2015). The progressive disturbance in soil associated with a rapid increase in world demand for food, pressure on agricultural systems to operate more intensively and efficiently in order to reduce environmental impacts. The increase in the level of diversity in the time and space of agricultural systems has been proposed as mechanisms to increase sustainability and resilience (Garrett et al., 2020).

Integrated Crop-Livestock Systems (ICLS) are the most promising alternative, based on sustainable intensification, of a system able of meeting the growing demand for food (FAO, 2011). ICLS are systems designed to exploit synergisms and emergent properties that result from interactions in the soil-plant-animal-atmosphere compartments on different spatiotemporal scales through the integration of crops and animals (Moraes et al. 2013).

Large herbivores play a dominant role in nutrient cycling in integrated systems modifying the flows between the soil-plant-animal compartments, by ingesting and consuming nutrients and changing the sward structure, before the nutrient return to the

system (Bardgett and Wardle, 2003). The deposition of animal manure strongly influences the concentration of nutrients and in the microbial communities, consequently, enhance the availability of N and the decomposition of organic matter (McNaughton, 1992).

The lower dependence on external inputs is a characteristic that results from greater efficiency in the use of nutrients in the ICLS. Regarding N, an increase in the availability of ammonium (+70%) corroborates a decrease in demand of N fertilizer (Carlos et al., 2020), which reduces market fluctuations, leading to a system with greater self-sufficiency (Franzluebbers, 2007; Garrett et al., 2017).

Italian ryegrass is an adapted grass species recommended to compose ICLS in lowland (Marchezan et al., 2005). Italian ryegrass is one of the most important temperate pastures in cropping systems during cool season in Brazil southern (Varella et al., 2010). Ryegrass is a high-quality, a high-producing forage grass, an excellent reseeded, and responsive to N fertilizer (Beck et al., 2020). N is the main factor limiting leaf development and radiation use efficiency in ryegrass (Viegas, 1998).

The nitrogen (N) is a key factor in the production of food, feed and fiber, thus characterizing an important tool to meet the demands of a growing population. In the 20th and 21st centuries, synthetic N fertilizers has contributed greatly to the increase in production needed to feed and treat the growing human population (Mosier et al., 2004).

The availability of N in the soil is generally an important limiting factor for the productivity of agricultural systems. Therefore, the utilization of fertilizer and legumes crops to supply N requirements is one of the fundamental factors for meet the food production to the growing demand of the human population (Angus, 2001).

A greater diversity of agricultural crops allows residues with different qualities to directly affect soil carbon and N stocks and microbial diversity and activity, ultimately impacting the entire nutrient cycling process (McDaniel et al, 2014). Crop residues with low C:N ratio associated with an increase in temporal diversity restores the biological link between the C and N and can lead to better balances global C and N (Drinkwater et al., 1998). The mineralization of N from waste and the organic N accumulated in the soil increase its supply for non-leguminous species that participate in crop rotation (Mielniczuk et al., 2003; Conceição et al., 2013).

Mixed cultures are believed to have a more efficient use of resources compared to monocultures (Stigter and Baldy 1995). Grass-legume mixtures are the most

common mixtures. Legume crops can improve N nutrition by a directly transfer and an indirectly contribution through N mineralization from nodules and plant tissue senescents.

The unpredictability in the supply of N according to the type of soil, management of the previous crop and climate, urges farmers to apply significantly higher amounts of N than the corresponding to maximum yield, which leads to greater costs and environmental pollution (Lemaire, Jeuffroy and Gastal, 2008). Even so, Lawlor, Lemaire and Gastal (2001) related that a large part of pastures does not receive N fertilizers in the world, which compromises the expression of the genetic potential of the crop.

The adjustment N inputs according to requirements of the crops corresponding to the target yield is a major challenge for farmers. A sufficient supply of N by fertilizers can provide a sufficient amount of N for the plants to reach the potential growth allowed by the amount of energy intercepted by the crop (Lemaire, 1997).

The recommendations of N fertilization are based on the soil organic matter (SOM) content (CQFS RS/SC 2004; 2016), because N content in the soil has a high correlation with SOM (Mello et al., 1989; Schulten and Schnitzer, 1997). However, the determination of N fertilization management based on SOM analysis may be inaccurate, considering the great instability of N in soil and dependence on the C/N ratio of organic matter, this recommendation promotes an incomplete measurement of N status (Sartor, 2009).

Plant-based diagnostic of N status is a valuable tool for making decisions on the need to apply a supplemental amount of N (Lemaire, 1997). The Nitrogen Nutrition Index (NNI) is an example of plant-based diagnostic based on the concept of critical N concentration (N_c), defined as the minimum shoot N concentration necessary to obtain maximum growth rate (Ulrich, 1952). Values of N_c are high at the start of the growing period and decline during growth, in relation to dry matter accumulation (DM, t DM ha⁻¹) representing a dilution phenomenon (Farrugia et al., 2004).

This study aims to evaluate the N nutritional status of Italian ryegrass in pure and mixed swards on different ICLS under different N levels.

2. LITERATURE REVIEW

2.1. The process of specializing complex systems

The transition from hunter-gatherers to farmers made by our ancestors, probably represents the most important turning point in our history, with enormous implications. Crop production and livestock were developed up to 8 to 10 millenia ago (Smith, 1995). A wider variety of products has been provided from these systems for farmer's family than any company alone. Besides, offered a means of using crop residues or noncultivated land to produce animal products, while generating manure to improve fertility and soil quality (Russelle et al., 2007).

The increasingly specialization in many industrialized countries corroborates the separation of crop and livestock enterprises in the last 60 years (Ray and Schaffer, 2005). A multiplicity of factors supported a higher genetic uniformity within crop species in agricultural systems, for instance, ease of access to mineral fertilizers and pesticides, concentration of improvement efforts on the most economically important crops and changes in agricultural policies that allow producers to respond more freely to market signals, incentives and technological changes (Fausti, 2015).

Diversified cropping systems have been replaced by one or a few species, usually genetically homogeneous, culminating on simplified specialization. This process of simplifying the structure of the environment over vast areas emerged from modern agriculture, replacing the diversity of nature with a small number of cultivated plants and domesticated animals. Agriculture has become highly specialized, especially in industrialized countries, in response to political and economic restrictions (Russelle et al., 2007; Hendrickson et al., 2008), leading to a drastic reduction in the number of establishments, while an increase in physical area and labor productivity (Hanson and Hendrickson, 2009). Diversity loss, environmental pollutions and fragmentation of "habitats" were consequences from productivism race (Lemaire et al., 2013).

The concerns about resilience, adaptability to climate change, multifunctionality of agricultural landscapes, provision of ecosystem services and biodiversity of cropping systems has raised due increasingly growth of environmental problems generated by simplifying systems (Rusch et al., 2016). The simplification of agricultural systems outcomes an artificial ecosystem that requires constant human intervention, whereas the internal regulation of function is a product of plant biodiversity through flows of

energy and nutrients, progressively lost in these conditions (Swift and Anderson, 1993).

The specialized intensification was accompanied by a massive mechanization on farm-level. Soil tillage was a technology inherited from industrialized countries in temperate climates regions, where soil tillage is commonly used to accelerate soil warming and water evaporation in the spring, incorporate surface materials, and temporarily improve soil physical conditions for plant establishment and growth (Nunes et al., 2018). When snows in these regions, soil freezing paralyzes microbial activity, as the most of plants growth, preventing the nutrient from being assimilated by soil organisms, leaving them free in their mineral form and susceptible to leaching after thawing (Assmann, 2017).

Conversely, tropical and subtropical regions, which had imported that philosophy, could verified the incompatibility with its edaphoclimatic characteristics. Tropical climates favor fast decomposition of soil organic matter (SOM), releasing into the soil organic composts in solid, liquid, and gaseous forms with variable compositions (Gmach, 2018). Soil tillage exposes organic residues to oxidizing conditions accelerating microbial activity, and then elevating the decomposition rate (Thönnissen et al., 2000). The acceleration of decomposition of organic matter promotes mineralization of nutrients. Then, nutrients are released in soil solution before a sufficient amount of plant roots to absorb them, thus leaving them susceptible to losses, especially N and K leaching (Assmann, 2017).

In Brazilian Southern, the continued implementation of these agricultural practices carried out SOM and nutrients depletion, making soils unproductive in the 1960s. The non-sustainability of these practices accelerated soil degradation, reduced its productivity and made it vulnerable to erosive processes (Anghinoni et al., 2013).

Economically and environmentally sustainable yields will only be achieved with the maintenance or restoration of soil health (Cardoso et al., 2013). Soil health has been defined as “the ability of a living soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health” (Doran et al., 1996, 1997).

Total organic matter/carbon is the most frequently indicator in a reviewed soil quality assessment approach (Bünemann et al., 2018). Sufficient SOM levels conservation is critical for biological, chemical and physical soil functioning in

temperate and tropical ecosystems. The function of organic matter is both direct and indirect, its direct role is concerned with the provision of plant nutrients via the processes of decomposition and mineralization and its indirect role is associated with its effect on the physicochemical properties of the soil (Campbell, 1978). Therefore, beyond ensure soil fertility, improve carbon (C) sequestration, reduces soil erosion and preserve soil biodiversity (Six et al., 2002).

N is the most important nutrient in SOM, considering the economic scope, since despite the same essentiality, C is available in the atmosphere at no cost (Allison, 1973). Assuming only more modest increases in cropland of 10–25% by 2050 (Schmitz et al., 2014), future demand for food requires greater production per unit of land to meet crop production with world demand for food, which will be achieve a improve in global N use (Tilman et al., 2011).

2.2. Nitrogen

N is an essential macronutrient that affects plant growth and development, given it is an important component of chlorophyll, amino acids, nucleic acids, secondary metabolites (O'brien et al., 2016) and other important biomolecules such as ATP, NADH, NADPH, proteins and numerous enzymes (Miflin and Lea, 1976; Harper, 1994). N nutrition increases metabolic processes that influence the physical-chemical environment at the soil-root interface, changes the conditions of the rhizosphere, interferes with the absorption of cations and anions and increases or represses the activity of various enzymatic systems (Fernandes and Rossiello, 1995).

The lithosphere is the biggest deposit of N, where it is distributed in rocks, at the bottom of the ocean and in sediments that contain 1×10^{23} g N, representing 98% of the existing N. The second largest reservoir of the element is the Earth's atmosphere, estimated in $3,9 \times 10^{21}$ g N. And thirdly, the biosphere contains an approximate an amount of $2,8 \text{ e } 6,5 \times 10^{21}$ g N (96% total terrestrial organic N in death organic matter and 4% in living beings form) (Moreira and Siqueira, 2016).

Although the lithosphere comprises the main N reservoir, it is the only essential plant nutrient that is not released by the weathering of minerals in soils, except possibly for small amounts of geogenic N (Schulten and Schnitzer, 1997). And even thought almost 79% of atmosphere gas volume is composed by dinitrogen form (N_2), a very limited number of microorganisms have the ability to use elementary N (Stevenson,

1965), because has very low reactivity in this form, not being able to react under natural conditions, unlike other diatomic molecules, such as O₂, NO or CO.

Dinitrogen is converted into usable forms by higher plants and animals by combining H₂ or O₂ in processes as biological fixation of N₂ by bacteria, atmospheric and industrial discharges, such as NH₃, NO₃ or cyanamide for the manufacture of commercial fertilizers (Muchovej and Rechcigl, 1994). Therefore, the processes that provide available N to the soil are fertilizer additions, symbiotic fixation, nonsymbiotic fixation, rainfall, organic matter (Scarsbrook, 1965) and plants and animals' wastes.

N can undergo many transformations in the soil and is the essential element that assumes a greater number of forms in the soil, specifically takes nine different chemical forms in the soil, corresponding to different oxidative states. All these transformations comprise the N cycle, whose complexity depends on the factors present in a specific system. N transformations is driven by chemical, physical and biological process within a specific environment (Boswell et al., 1985). Processes driven by microorganisms, such as N fixation, nitrification and denitrification constitute most of the transformations of N and play an essential role in the N fate in Earth's ecosystems (Robertson and Groffman, 2015).

N is of great importance in limiting global primary production, but offers a potential impact on human and environmental health. The widespread adoption of N fertilizer provided considerable gains to society. It is estimated that 40% of the protein consumed globally by humans originated from the N supplied through fertilizers (Smil, 2001). Lamentably the recovery of N by plants often corresponds to less than 50 to 60 percent of the applied N (Balasubramanian et al., 2004) fundamentally due to the mobile nature of element and inadequate management practices.

The N can be lost from the site of application through soil erosion, runoff, or leaching of nitrate or dissolved forms of organic N or through gaseous emissions to the atmosphere in the forms of ammonia, N oxides, nitrous oxide or dinitrogen (Goulding, 2004).

The net balance between the processes of mineralization, immobilization, nitrification, leaching, volatilization and denitrification determinates the availability of N in the soil. However, from the quantitative point of view and management practices, the processes of mineralization and immobilization of N are those that most influence the availability of N for crops (Ferreira et al., 2016). These processes are primarily

controlled by organic matter quality, specifically the availability of C in the material relative to its available N (Robertson and Groffman, 2015).

A sequence of processes of uncoupling and re-coupling of C-N driven by soil microorganisms allows the fresh organic matter decomposition. The C/N ratio of 20-50 g g^{-1} gradual decrease of about 10 g g^{-1} , leading in more stabilized compounds (Soussana and Lemaire, 2014).

The microbes' protoplasm has a N content that varies between 3 and 12%, which is often higher than the decomposing substrate. Mineralized N will be used to form this protoplasm (Harmsen And Kolenbrander, 1965). A critical value of mineralization/immobilization was established in 25 g g^{-1} . Therefore, if the substrate has a higher C/N ratio (above 25 g g^{-1}), the microorganisms will reabsorb all mineralized N and no inorganic N will accumulate, thus a depletion of N can induce a N deficiency. On other hand, under low C/N ratio conditions (e.g. below 25 g g^{-1}), the net immobilization of N decreases and the mineralization process initiates (Manzoni et al., 2008).

The cropping systems under no-tillage (NT) and with different crop rotations results in different additions of plant residues in soil, which can cause differences in total N stocks, mainly in the particulate fraction, as seen in the NT system with cover crops and grain production (Lovato et al., 2004; Bayer et al., 2006).

The greater use of legumes and the implantation of species with greater biomass production cause greater storage of total N in the soil (Bayer and Mielniczuk, 1999). The contribution to re-coupling the C and N cycles in agroecosystems and increase N reservoirs in the soil and improve the N use efficiency at rotation level are the most widespread benefit of legume crops utilizations (Drinkwater et al., 1998; Dabney et al., 2001; Peoples et al., 2009)

The Symbiotic N fixation is influenced by abiotic factors, as soil inorganic N content, soil moisture and soil temperature and biotic factors, as genotype, seeding rates, defoliation height and intensity are of decisive importance (Hogh-Jensen and Schjoerring, 1994; Peoples et al., 2001). Due to these variations the amount of N fixed and transferred is very variable. Louarn (2015) report a N transfer from white clover to the associated ryegrass up to 72 $\text{kg ha}^{-1} \text{y}^{-1}$. Sincik and Acikgoz (2007) found a transfer of 41 $\text{kg ha}^{-1} \text{y}^{-1}$, under unfertilized conditions, from white clover to perennial ryegrass.

The absorption of NO_3 by the roots depends on the concentration of NO_3 in the soil solution, on the volume of soil explored by the roots, on the root density and on the efficiency of the roots in the absorption of NO_3 (Engels and Marschner, 1995). The efficiency of roots in uptake is affected by metabolic demand and conditions such as temperature (Macduff et al., 1987). The explanation for the difference between the rates of N transferred between legume species may be the differences between the legumes in their growth habits, root characteristics, nodulation profiles, root exudation profiles, decomposition rates and mycorrhizal association (Thilakarathna et al., 2016).

Given its importance and unpredictability, it is well recognized that N deeply influences plant growth, development, and yield in crop plants. The challenge of matching N requirements that makes N the most difficult of the essential elements to supply in sufficient quantities for plant growth is that the N compounds available to the plant are also those most easily made unavailable to plants, either temporarily due to the fixation and accumulation in the soil or completely lost from the biosphere by leaching, denitrification and volatilization.

A classic example of specialized systems under conventional management is the rice-based cropping system, that occurs predominantly in an environment called lowlands.

2.3. Lowlands

Lowland are agronomically characterized by altitude of up to 50 meters and declivity of up to 5%. It's estimated to occupy 7 to 9 million km^2 , about 4 to 6% of the earth's surface (Lefeuvre and Bouchard, 2002). Lowland's soils are described by high susceptibility to saturation, due to their position in the relief and their physical characteristics, what results in water accumulation on the soil during the growth of annual crops (Brinkman and Blokhuis, 1986).

Most of Brazil's lowlands are concentrated in the Southern region, mostly in Rio Grande do Sul (RS) State. The peculiar characteristics of these soils, especially the low hydraulic conductivity, makes their management extremely complex and restrict the introduction of some upland species (Scivittaro and Gomes, 2007). However, associated with its edaphoclimatic conditions becomes extremely favorable to the cultivation of flooded rice (*Oryza sativa* L.). For this reason, 3 million (M) of the 5.4 M hectares (ha) of lowland in RS, are designated for flooded rice cultivation. However,

only a third are cultivated annually, mainly due to water availability restriction (Pinto et al., 2004; SOSBAI, 2018).

The cultivation of flooded rice is historically characterized by monocropping with winter fallow period and is strongly linked to practices of intense and frequent soil tillage, called the conventional system (SOSBAI, 2018). The fallow period, a practice carried out in many lowland rice fields during cool season, causes low nutrient cycling through spontaneous vegetation and, consequently, the contribution by soil mineralization is very low (Borin, 2018), especially due predominance of sandy soils, with low organic matter content in all rice regions of RS State (Boeni et al., 2010).

The recurrent use of revolving practices promotes soil disturbance, considered the main cause of soil degradation worldwide (Lal, 2015). A greater soil degradation in long-term experiments with soil disturbance in flooded rice systems was related to lower pH, cation-exchange capacity (CEC), and nutrient use efficiency (NUE) (Flinn and De Datta, 1984). Cassman et al., (2005) report a yield decline in most continuous flooded rice long-term experiments, associated with a decrease in the effective N supply from although total soil N remains constant.

The progressive and drastic decrease in SOM explains why even with high levels of fertilizers added annually there is no increase in its contents in the soil. Monoculture rice areas with lack of crop rotation and fallow in the fall/winter period converge to reduce soil fertility levels and require high inputs of mineral fertilizers to obtain satisfactory yields of irrigated rice (Carmona et al., 2016). According to Silva and Schoenfeld (2013), impoverishment of the soil is not due to the lack of nutrient input, but to the lack of crop rotation and adequate management practices. The authors found that after 8 consecutive harvests of irrigated rice, with a total addition of 1,200, 480 and 800 kg ha⁻¹ of N, P₂O₅ and K₂O, respectively, the levels in the soil of P and K decreased, while those of SOM remained the same.

Financial exhaustion related to rice price and high labor, water and energy costs (Kumar and Ladha, 2011) associated with soil impoverishment, confirm that increasing productivity in rice has been based on non-sustainable intensification, with a high degree of vulnerability (Moraes et al., 2014). Meanwhile, a significant increase in food demand, projected to double by 2050 (Tilman et al., 2011) corroborates the urgent needed for adoption of sustainable intensification (SI) based on conservative management practices in rice cropping systems.

Agricultural sustainability is based on the principle of meeting the demands of the present without compromising the ability of future generations to meet their own demands. Thus, long-term management of natural and human resources is equally important as short-term economic gain (Brodthorn and Six, 2011).

Pretty and Bharucha (2014) defined SI as “a process or system which the increase in agricultural yields is reached without adverse environmental impact and without the conversion of additional non-agricultural land”. The SI is the answer for the call to increase food production on existing land in a way that puts much less pressure on the environment and does not impair our capacity to continue producing food in the future (Garnett et al., 2016).

According to Pretty (2008) the key principles for sustainability are: (i) integrate biological and ecological processes such as nutrient cycling, N fixation, soil regeneration, allelopathy, competition, predation and parasitism into food production processes, (ii) minimize the use of those non-renewable inputs that cause harm to the environment or to the health of farmers and consumers, (iii) make productive use of the knowledge and skills of farmers, thus improving their self-reliance and substituting human capital for costly external inputs, and (iv) make productive use of people’s collective capacities to work together to solve common agricultural and natural resource problems, such as for pest, watershed, irrigation, forest and credit management.

2.4. Integrated Crop-Livestock Systems

Integrated Crop-Livestock Systems (ICLS) are the most promising alternative to reach the increasing food demand, based on SI principles (FAO, 2011). ICLS are systems that integrate crop and livestock production activities on different spatiotemporal scales, designed to exploit synergisms and emergent properties that result from interactions in the soil-plant-animal-atmosphere compartments (Moraes et al., 2014). The great innovation of these millenia systems is the insertion of conservationist agricultural principles. Reduced or NT, soil-cover requirements and, diversification of cropping systems, associated with the effects of grazing, interact synergistically and contribute to emergent properties (Anghinoni et al., 2013).

Cover crops has many agronomic benefits, but its adoption seems to be limited, due to investment without immediate financial return. The insertion of grazing animals

in these environments can provide immediate economic benefit to farmers, especially with the development of conservation tillage technologies to prevent deterioration of soil and water quality (Franzluebbers, 2007). This author indicates some reasons for the transition from specialized systems to ICLS: (i) specialized farms operating on marginal profit, (ii) economic vulnerability with specialized production, (iii) high cost of fuel and nutrients, (iv) pests becoming more damaging with monocultures, (v) yield decline due to long-term management-induced constraints on soil chemical and physical characteristics and biological diversity, (vi) spatially and temporally improved nutrient cycling on a field and landscape level with integration of enterprises, and (vii) conservation of soil and water resources with greater adoption of sod-based management approaches.

Integration can be both on-farm and areawide basis, which may involve some specialization (FAO, 2011). Bell and Moore (2012) define integration at four levels: synchronized, rotated, segregated or specialized. According to these authors, at synchronized level, (e.g., activities occur simultaneously or in the same cycle) the maximum benefits of ICLS are obtained, best use of nutrients, less inputs per product produced, greater efficiency in the use of machinery and labor, greater profitability through both the increase in income in the same area unit, and the reduction the risk of agricultural activity.

The components arrangements are involved in the three proposed dimensions, that transcend the current limits and the nature required for their study is transdisciplinary, then, emerging properties are created, as effects described so far are not simply cumulative. These emerging properties are not easy to observe, predict or even prove (Anghinoni et al., 2011). Despite that, scientific community recognizes that integrated systems are efficient in cycling nutrients and energy (Entz et al., 2005), more sustainable (Ryschawy et al., 2012), resilient (Lemaire et al., 2014; Peterson et al., 2020) and has more stability (Albuquerque et al., 2021).

The development of many studies regarding nutrients NUE allowed an advance in knowledge regarding nutrient cycling dynamics. This deeping of knowledge conceived a new fertilization concept, called system fertilization, which considers the systems as a whole, seeking maximum efficiency in the use of nutrients. System fertilizer approach is based on biological cycling of nutrients between the phases of a rotation

system, considering the transfer of fertilization between all crops involved in rotation/succession in a cropping system (Assmann et al., 2017).

The system fertilization consists of anticipating the application of fertilizers of summer crops in pasture within the objective of apply the fertilizer in the period of less export and greater nutrient cycling via animal consumption. The point is that N export is 320, 150 and 175 kg ha⁻¹ for the production of 4, 10 and 10 t ha⁻¹ of soybean, rice and corn, respectively. Whereas for the production of 450 kg ha⁻¹ of meat, exportation is only 11 kg ha⁻¹ (CQFS RS/SC, 2016; Ball et al., 1991).

Pasture fertilization can optimize fertilizer use, determined by nutrient (re) cycling through grazing, consequently improving soil fertility (Carvalho et al., 2010). The greater NUE in forage crops occurs because the ruminants export little amounts of nutrients via animal tissue, returning 70-95% of them from the plant to the soil, through excrement (Russelle, 1997). Studies in Brazil confirmed the viability of this concept in subsequence crop yield, as corn (Assmann et al., 2003; Sandini et al., 2011; Bortolli, 2016), soybean (Denardin et al., 2020a; Farias et al., 2020) and rice (Denardin et al., 2020b).

Forage crops that precede crops in ICLS plays a multifunctional role: 1) provide food to grazing animals and contributes by increasing income through diversification of activity; 2) offers ecosystem services, optimizing the use of resources available in the environment, reducing nutrient losses, increasing the accumulation of dry matter and, consequently, carbon (C) sequestration; and 3) transfer matter and energy to succession crop, specially nutrients released from litter and manure decomposition, involving a complex nutrient cycling system, etc.

The roots of forage plants are essential binding agents, that help in the development of a stable soil structure and rich in porosity, which benefits the infiltration of water in the soil profile, thus improving the flow of water to the groundwater reservoirs and consequently decreasing the possibility of erosion caused by runoff (Franzluebbers et al., 2014). This almost constant presence of plant over the soil causes nutrients to be constantly absorbed by plants and to be linked to organic compounds, thus reducing the chances of their loss (Assmann, 2017).

Large herbivores play a dominant role in nutrient cycling in pastures, since they change the speed and the route between the compartments via forage consumption, digestion and subsequent return to the system, acting as a catalyst that makes the

cycle of plant material and profoundly modifies the dynamics of nutrients in the various compartments of the system (Anghinoni et al., 2011). The grazing promotes a continuous growth, consequently, prolonged demand for nutrients, thus being able to contribute to the mitigation of its losses, and this occurs due to the synergism between root growth and partial thinning of the plants leaves that stimulate regrowth (Moraes et al., 2014).

The magnitude of the animals' interference in nutrient cycling will depend on the distribution of excreta in the pasture, the area affected by the excreta and their nutrient content. The deposition of animal manure strongly influences the concentration of nutrients and in the microbial communities, consequently, enhance the availability of N and the decomposition of organic matter (McNaughton, 1992).

The ability to achieve a greater extent of nutrient cycling and a more rapid distribution of limestone at depth combined with a greater accumulation of shoot and root dry matter in an ICLS under NT conditions with moderate grazing relative to non-ICLS expresses an important property of the system's efficiency regarding the use of nutrients. Denardin et al. (2020b) showed that flooded rice under ICLS yields more grain while requiring lower fertilizer application than conventional system (CS) and has higher NUE compared with CS.

Grazing is the cause and consequence of the sward structure of the pastoral environment (Carvalho, 2013). By altering the sward structure, interferes in N content in the plant tissues and, also, with the incorporation of organic matter in the soil due to animals trampling, therefore grazing changes the N cycle. These changes caused by grazing tend to increase the availability of inorganic N, improving the quality of plant biomass (Bardgett et al., 1998), due to the effect on microbial immobilization and the alteration of the C flow from plants to soil (Stark and Grellmann, 2002).

Moderate grazing increases the accumulation of C in the soil, since the total forage production in these conditions is higher (Carvalho et al., 2011). Furthermore, crop development is influenced by pasture management conditions (Carvalho et al., 2010), by the better quality of pastures and feces. Thus, at moderate grazing intensities, greater amounts of N are released via pasture residues and excreta, in relation to the high grazing intensity (Assmann et al., 2015). Soil aggregation is greater under moderate grazing conditions, and other physical, chemical and biological soil attributes are improved (Carvalho et al., 2010).

The choice of components and strategy of its spatio-temporal arrangements, defines the elements involved, described as the nature and the number of flows, as the magnitude, from current biogeochemical cycles (Anghinoni et al., 2013). The degree of entropy of this system generates a new state of order and a new degree of connectivity between components and multiple interactions that overlap with the simple relationships previously experienced, from which new systemic processes emerge (Vezzani and Mielniczuk, 2009).

2.5. Cropping systems diversification

Kremen et al. (2012) refer to a farming system as “diversified” when it intentionally includes functional biodiversity at multiple spatiotemporal scales, through practices developed via traditional and/or scientific agroecological knowledge. Moreover, they argue that the fundamental difference between diversified farming systems and sustainable, organic, multifunctional and ecoagriculture systems is its premise in farming practices design to support functional biodiversity across spatial and temporal scales, the necessary ecosystem properties providing critical inputs to agriculture are supplied.

Hufnagel et al. (2020) define diversification as “a process that makes simplified farming systems more diverse in time and space, adding additional crops”, where common examples for crop diversification are crop rotations, double cropping or intercropping, etc. The concepts of diversity and diversification are commonly confused in scientific community which promotes a high variability of approaches related to diversification of cropping systems. According to these authors, “while the former deals with biological principles such as genetic diversity, the latter deals with agronomic principles such as crop rotation or mixed cropping that subsequently might lead to higher biodiversity and associated ecosystem services. Diversification is the process that leads to the state of diversity. Depending on the initial situation, the same measure of crop diversification might lead to completely different states of biodiversity or ecosystem services”.

Biodiversity provides essential ecological services, which can enable the sponsoring soil fertility of agroecosystems, protection and the productivity of their crops, pest regulation by restoring natural control of insect, disease and nematode pests and also produces optimal nutrient recycling and soil conservation by activating

soil biota, all factors that lead to sustainable yields, conservation of energy and less dependence on external inputs (Altieri, 1999).

The cultivation of a sequence of plants species on the same land is called crop rotation (Yates, 1954). The crop rotations practices date back to antiquity (Bullock, 1992) and the discovery that legumes can fix atmospheric N at the end of the 19th century was one of the main reasons for rotations maintaining popular at the early 20th century. Fundamentally because, at that time, the only known way to add N to the soil was by adding manure (Karlen et al., 1994).

The biological diversity of crop rotation can be divided into temporal and spatial components. Temporal diversity results from a sequence of crops in a given field, which characterizes an important tool in breaking pest cycles, reducing soil erosion, and increasing yields. Whereas spatial diversity results from greater numbers of crops being grown at a given time on the landscape (Karlen et al., 1992).

The monocultures' crops rotation creates temporal rather than spatial biodiversity. Increasing the biodiversity of temporal plants can provide some of the same underground benefits as spatial diversity, such as positive effects on soil C and N (McDaniel et al., 2014). Crop rotations plainly increase soil microbial biomass (Moore et al., 2000) and activity, and it seems to facilitate microbes in enhancing soil N supply to crops (Mc Daniel et al., 2016). Rotation length, particularly the inclusion of forage crops, positively affected organic C (Karlen et al., 2006).

The greater richness of agricultural crops grown in a diverse environment impact the entire nutrient cycling process, since allows waste with different qualities to directly affect soil C and N stocks and microbial diversity and activity (McDaniel et al., 2014). The activity of the soil microbiota reflects all changes in the order of energy and nutrient flows generate by pastures, no-tillage and crop rotation. The contribution of the high input of plant residues with high root density promotes the increase of biomass and microbial enzymatic activity in the soil under ICLS (Martins et al., 2017).

According to Drinkwater et al. (1998) the composition of plant species and the quality of litter significantly influence SOM turnover and promotes a greater C and N retention. Low C:N residues combined with increased temporal diversity restores the biological link between the C and N and can lead to better balances global C and N. However, if the amount of energy and aggregate of litter is not sufficient to supply the requirements of biota, binders and particulate organic matter will be used for

microorganisms as a source of energy and C, and the complex structures already formed will be destroyed (Vezzani and Mielniczuk, 2011).

Many authors studying nutrient cycling have quantified the N content from the plant residue. Assmann et al. (2015) observed that soybean leaves through the residue of its shoot an amount of residual N on average 46 kg ha⁻¹, which are released gradually over the pasture cycle that will come in succession of ICLS with soybean-pasture rotations. Toomsan et al. (1995) found that soybean contributed 15 kg N ha⁻¹ to the total benefit of residual N. Heichel and Barnes (1984) found that an estimated value of 24 kg N ha⁻¹ returned to the soil, when 90% of N is biologically fixed.

2.6. Nitrogen nutrition of ryegrass in pure and mixed swards.

The traditional rice-based cropping system in lowland southern Brazil is combined with extensive and inefficiency livestock production in RS. According to Reis (1998), the animals' feed is based on natural vegetation and a succession field, culminating in a low rate of livestock production, considering that cool season (autumn-winter) is the most critical period due the scarcity and low quality of food supplied to the animals.

Italian ryegrass is an adapted grass species recommended to compose ICLS in lowland (Marchezan et al., 2005). It is estimated that at least 30% of the 7 M hectares of cultivated pasture is occupied by Italian ryegrass in Brazil southern, becoming one of the most important temperate pastures in cropping systems during cool season (Varella et al., 2010). Ryegrass is a high-quality, a high-producing forage grass, an excellent reseeder, and responsive to N fertilizer (Beck et al., 2020).

N is the main factor limiting leaf development and radiation use efficiency in ryegrass (Viegas, 1998). Under non-limiting N supply, it can be used in autumn with very high forage production levels, since incident solar radiation and temperature do not limit growth (Nabinger et al., 2000).

Many authors (Kemp, 1974; Marino et al., 2004; Lippke et al., 2006) report that ryegrass yield generally increases with N fertilizer application rates. But variation in soil N supply and crop N demands from location, year and season promotes a fluctuating response of forage grasses to N fertilization (Whitehead, 1995). Heringer and Moojen (2002) verified a quadratic relationship between N fertilization on forage production in the studies consulted. Varella et al. (2010) found an indication that N uptake of ryegrass could continue to increase above 200 kg N ha⁻¹ in southern Brazil. While

Lazenby (1981) showed that the response of forages to N fertilization increasing up to 300 kg ha⁻¹ for temperate grasses.

Multispecies agricultural systems can often be seen as a practical application of ecological principles based on biodiversity, plant interactions and other natural regulatory mechanisms. Even though they are sometimes considered more difficult to manage, they are considered to have potential advantages in productivity, production stability, resilience to interruptions and ecological sustainability (Vandermeer, 1989).

A sustainable alternative to increase the N nutrition of ryegrass is through mixtures with legumes, due the recognized in reducing the N fertilizers use (Walker et al., 1954; Simpson 1965), therefore is a mean to improve N use efficiency (Corre-Hellou et al., 2006; Bedoussac and Justes 2010).

Pastures based on legumes accumulate SOM, improve soil fertility resulting in enhance N availability (Greenland, 1971) and CEC (Russell, 1961). The increase in the SOM of legumes in crop rotation can occurs directly by adding C from plant tissues, or indirectly, by changing its quality and rotation, even as affecting the amount of residue produced by the subsequent crop, in function of the incorporation of symbiotically fixed N (Cadish et al., 1998).

Among the various benefits obtained with the use of mixed cultures, the main one reported is the greater resources use efficient than monocultures (Stigter and Baldy 1995). Regarding water and light use efficient, it can be explained by the different exploration in volumes of air and soil of exchange organs above and belowground from associated species and therefore, different ecological niches (Vandermeer, 1989).

Regarding N scopes, its more superficial location in the soil layer would provide a more direct competition between the mixtures, however the greater efficiency of its capture and use stems from the fact that the most common mixtures consist in grass and legume mixtures, culminating in the utilization of two different N sources: soil absorption by grass and legumes and symbiotic N₂ fixation from the atmosphere by legumes (Cruz and Soussana, 1997).

The benefit for the grass associated in legume-grass mixtures comes from the lower competitive ability to capture N from soil by the legume (Soussana et al., 1989; Faurie, 1994), which increases the N availability for grasses (Vallis et al., 1967).

Legumes provide N directly through association of arbuscular-mycorrhizal fungi (Bethlenfalvay et al., 1991; Frey and Schiepp, 1992; Johansen and Jensen, 1996) and

leaf leachates or root exudates N containing (Paynel et al., 2001), and indirectly by N mineralization from nodules and above- or belowground plant tissue senescents (Høgh-Jensen and Schjoerring, 2001; Ledgard and Steele, 1992; Tobita et al., 1994).

The net amounts of N transferred from legumes to the associated grasses may vary from not significant (Walker et al., 1954) to highest reported levels of 75-110 kg N ha⁻¹ y⁻¹ (Elgersma and Hassink, 1997; Høgh-Jensen and Schjoerring, 2000), which represent a highly variable amount. Besides the previously mentioned factors that interfere the N fixation process, the transfer of N is mainly affected by the N inputs. The grass and legume proportion in biomass legume have been proposed to explain differences between sites, species or cultivars (Ta and Faris 1987; Heichel and Henjum 1991; Schipanski and Drinkwater 2012) in turn, are highly variable according to the N addition.

2.7. Nutritional Nitrogen Index and dilution curve

To ensure the potential growth, allowed by the amount of energy intercepted by the crop yield, the N inputs are often higher than the minimum required for maximum crop growth, fundamentally because N fertilizers are relatively cheap compared to the expected economic benefits from a maximized crop yield (Lemaire, 1997). Excess N fertilizer promotes potential N losses to the environment through leaching and denitrification (Bélanger and Gastal, 1999). Besides that, excessive doses of N can cause toxicological problems for animals due to excess nitrate accumulation in plant tissues (Jönck et al., 2013).

Nevertheless, Lawlor, Lemaire and Gastal (2001) diagnosed that, worldwide, a large part of pastures does not receive N fertilizers. The reduction of N fertilization decreases the growth rate of the forage, the density and height of the tiller and, finally, the mass of the forage (Wilman, 1980). The lack of N limits the expression of the genetic potential of the forage, considering the pattern of response curves related previously.

About 95% of total soil N is closely associated with SOM, especially humic substances, that act as a deposit and supplier of N for plant roots and microorganisms (Schulten and Schnitzer, 1997). Plant uptake of soil N is correlated with particulate organic matter carbon (POM-C) (Nissen and Wander, 2003). The accumulation of decomposed and recalcitrant SOM pools provides a big reservoir of N that influences

its availability in the short time (Drinkwater and Snapp, 2005). Therefore, the maintenance of organic matter contributes to a constant permanence of N in the soil, which is temporarily immobilized by decomposing microorganisms in the soil and will be available to plants later (Korndörfer et al., 1997).

This close relationship with organic matter leads to recommendations for N fertilization based on the organic matter content of the soil (Mello et al., 1989). However, given that the availability of N is unstable and depends a lot on the C/N ratio of organic matter, this recommendation may be inaccurate (Sartor, 2009).

The adjustment of optimal fertilizer must consider the crop requirements and the soil N supply. However, both are difficult to predict because of their dependence on soil and climatic conditions (Bélanger and Gastal, 1999) and due to our knowledge gap about the basic mechanisms which govern N cycling.

The possibility of diagnosis of the N status of any crop during its development is a valuable tool for making decisions about N fertilizer and decreasing these inputs (Lemaire, 1997). An important tool for a diagnosis of the nutritional status of plants is the Nitrogen Nutrition Index (NNI), proposed by Salette and Lemaire (1981), confirmed in studies of N dilution curve (Lemaire and Salette, 1984).

The nutritional diagnosis of plants can be interpreted in its simplest terms as a study of the relationship between the nutrient content of the plant and its growth (Ulrich, 1953). The crop demand rate for N is the product of the potential growth rate (under non-limiting N supply) and the fraction of N in the plant necessary to allow maximum growth (Greenwood, 1982).

Nutrient requirement is not constant during the plant vegetative growth cycle. In early stages of regrowth, after defoliation, the plant requires a high N supply to support leaf area development and photosynthesis. As the proportion of the low-N structural components in the forage increases (Caloin and Yu, 1984), N requirement per unit of incremental DM decreases (Greenwood et al., 1990).

The minimum N concentration in shoots required to achieve maximum crop growth and yield is called N critical concentration (% N_c) (Ulrich, 1952; Greenwood et al., 1990). The concept of a critical N curve based on the N concentrations was first developed for tall fescue (*Festuca arundinacea*) in Europe by Lemaire and Salette (1984).

Lemaire and Salette (1984) demonstrated that for grasses growing with non-limiting N supply, the N concentration of a sward can be related to the dry matter accumulation by a simple equation (1):

$$\%N_c = aW^{-b} \quad (1)$$

Where W is the weight of aboveground biomass in $t\ ha^{-1}$ and $N_c\%$ is the N concentration as a percentage of dry matter. The coefficient “a” represents the plant N% for an aboveground biomass of $1\ t\ ha^{-1}$ and the coefficient “b” characterizes the pattern of decrease of N% during regrowth (Lemaire, 1997).

The coefficient a can vary between 3.6 and 4.8, for C4 and C3 species, respectively, due to different metabolic routes for CO_2 assimilation and differences in leaf anatomy (Brown, 1978, 1985; Field and Mooney, 1986). The coefficient b (fractional decline in N% per unit of aboveground biomass increase) only varies between 0.32 and 0.44 for a large number of species. Such approach was developed for several crop species, including temperate grasses (Lemaire and Gastal, 1997):

$$\%N_c = 4.8W^{-0.32} \quad (2)$$

The critical concentration of N in the plant decreases as plant grows, reflecting an ontogenetic plant architecture development leading to a dilution of N compounds. The dilution of N is the process corresponding to the faster accumulation of N-free compounds than the N compounds within the plant as it grows, leading to a decline in the N concentration of the plant with the accumulation of plant mass (Greenwood and Barnes, 1978; Lemaire, 2015).

These critical N dilution curves can be used to determine the N demands of plants and calculate the NNI that quantifies the N status of plants (Lemaire, Gastal and Salette, 1989; Lemaire and Meynard, 1997). At a given time, for a growing period of a crop, characterized by an actual crop N uptake (N_a) corresponding to an actual crop mass (W), it is possible to determine a NNI as the ratio between N_a and the critical N uptake (N_c) (Lemaire, 2015).

Currently no approach is valid for assessing the N nutritional status of mixed crops, considering the full range of possible mixtures explored by farmers (Louarn, 2021). However, Corre-Hellou (2005) proposed the NNI_{comp} index (3) for mixed

species. The NNI_{comp} assumed the hypothesis of a perfect asymmetry in the interaction between species (e.g. growth and N content of the component species not affected by its neighbour), which is equivalent to the situation of a taller very dominant species (Schwinning & Weiner, 1998), as Italian ryegrass in this study.

$$\text{NNI}_{\text{comp}} = \%N_i / \%N_c \quad (3)$$

Values of NNI close to 1 indicate that the crops were in situation of non-limiting N supply, values greater than 1 indicate a luxury consumption of N and values less than 1 indicate an N deficiency (Lemaire and Meynard, 1997).

The early diagnosis of N deficiency can predict the productive capacity of the pasture, as well as estimate N fertilization rates (Agnusdei et al., 2010). Situations in which the NNI is greater than or equal to 1.0, the amount of N applied can be delayed or decreased. Whereas when the NNI is less than 1.0, it is possible to anticipate the application, or even increase the N rates, depending on the intensity of this deficiency (Lemaire; Jeuffroy; Gastal, 2008).

3. HYPOTHESIS

3.1. An increase in cultivated plant diversity in rice-based ICLS improves nitrogen nutrition index (NNI) of Italian ryegrass.

4. OBJECTIVE

4.1. The objective of this study was to evaluate N status of Italian ryegrass under N levels and diversity of plants grown in rice-based ICLS.

CHAPTER II

NITROGEN NUTRITION OF ITALIAN RYEGRASS UNDER LEVELS OF DIVERSIFICATION IN RICE-BASED INTEGRATED CROP-LIVESTOCK SYSTEMS

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ABSTRACT

Integrated crop-livestock systems (ICLS) can be an alternative to increase soil N and C stocks and microbial activity, improving nutrient cycling through rotations of plants and insertion of grazing animals. The level of spatio-temporal diversification of plant arrangements and animal activity alters N flows in the system and therefore affects the supply of N to the plant. We aimed to assess the nitrogen status of Italian ryegrass pasture under different levels of cultivated plant diversity in rice-based integrated crop-livestock system (ICLS) using the Nitrogen Nutrition Index (NNI) as a diagnostic tool. The experimental design was conducted as a split-plot randomized complete block design with three replications. Treatments were a factorial of four ICLS (main plots) and three N rates (subplots). The ICLS represents different spatio-temporal variability of plants arrangement based on flooded rice: S2) Italian ryegrass under grazing (*Lolium multiflorum* Lam.) following a rice monocropping (*Oryza sativa* L.); S3) Italian ryegrass under grazing following a rotation of rice and soybean (*Glycine max* (L.) Merr); S4) Italian ryegrass and persian clover (*Trifolium resupinatum* L.) mixed under grazing following a rotation of rice, Sudan grass [*Sorghum × drummondii* (Steud.) Millsp] under grazing, soybean and corn (*Zea mays* L.) and; S5) Italian ryegrass, persian clover and birdsfoot trefoil (*Lotus corniculatus* L.) mixed under grazing following a 3-year rice succession field. The three N levels applied on winter forage crops were: a) no N application (N0), b) 150 kg ha⁻¹ (N150) and c) 300 kg ha⁻¹ (N300). The NNI approached the critical N (Nc) at the N150 level (0.94). While at the N300 level, the points surpassed the Nc in all systems (1.14) except for the S5 (0.97), characterizing a “luxury” consumption. All systems showed N deficiency at the N0 level (0.44), however the systems with mixed legumes (S4 and S5) obtained greater NNI of the ryegrass, but that was not statistically significant. In these field conditions, there is an indication that the N levels corresponding to the Nc for Italian ryegrass are between 150 and 300 kg⁻¹ N ha⁻¹. The S3 showed a greater NNI value at both, N150 and N300 levels, which determined its average superiority ($p = 0.0276$). The increase in the spatio-temporal diversification of the cultivated plant species in ICLS promotes a greater NNI, the S3 and S4 presented the highest NNI.

Keywords: diversification, biological cycling, N fluxes, sustainability, ecosystem services, resilience

NUTRIÇÃO NITROGENADA DE AZEVÉM ITALIANO SOB NÍVEIS DE DIVERSIFICAÇÃO EM SISTEMAS INTEGRADOS DE PRODUÇÃO AGROPECUÁRIA BASEADOS EM CULTIVO DE ARROZ

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RESUMO

Os sistemas integrados de produção agropecuária (SIPA) podem ser uma alternativa para aumentar os estoques de N e C e a atividade microbiana no solo, melhorando a ciclagem de nutrientes por meio de rotações de plantas e da inserção de animais em pastejo. O nível de diversificação espaço-temporal dos arranjos vegetais e da atividade animal altera os fluxos de N no sistema e, portanto, afeta o fornecimento de N para a planta. Nosso objetivo foi avaliar o status de nitrogênio (N) do azevém sob diferentes níveis de diversidade de plantas cultivadas em sistemas integrados de produção agropecuária (SIPA) à base de arroz utilizando o Índice de Nutrição de Nitrogênio (INN) como ferramenta de diagnóstico. O delineamento experimental foi conduzido como um delineamento de blocos completos casualizados em split-plot com três repetições. Os tratamentos foram um fatorial de quatro SIPA (parcelas principais) e três níveis de N (subparcelas): S2) Azevém (*Lolium multiflorum* Lam.) sob pastejo após uma monocultura de arroz (*Oryza sativa* L.); S3) Azevém sob pastejo após rotação de arroz e soja (*Glycine max* (L.) Merr); S4) Azevém e trevo persa (*Trifolium resupinatum* L.) misturados sob pastejo seguindo uma rotação de arroz, capim Sudão [*Sorghum x drummondii* (Steud.) Millsp] (sob pastejo), soja e milho (*Zea mays* L.) e; S5) Azevém, trevo-persa e cornichão (*Lotus corniculatus* L.) misturados sob pastejo após um campo de sucessão de arroz de 3 anos. As três doses de N aplicadas nas forrageiras de inverno foram: a) sem aplicação de N (N0), b) 150 kg ha⁻¹ (N150) e c) 300 kg ha⁻¹ (N300). O INN se aproximou do N crítico (Nc) no nível N150 (0.94). Já no nível N300, os pontos superaram o Nc nos sistemas (1.14), exceto no S5 (0.97), caracterizando um consumo de “luxo”. Todos os sistemas apresentaram deficiência de N no nível de N0 (0,44), porém os sistemas com leguminosas misturadas (S4 e S5) obtiveram maior NNI do azevém, mas isso não foi estatisticamente significativo. Nessas condições de campo, há indicação de que os níveis de N correspondentes ao Nc para o azevém italiano estão entre 150 e 300 kg⁻¹ N ha⁻¹. O S3 apresentou maior valor de INN nos níveis N150 e N300, o que determinou sua superioridade média (p = 0.0276). O aumento da diversificação espaço-temporal das espécies de plantas cultivadas no ICLS promove um maior INN, o S3 e S4 apresentaram os maiores INN.

Palavras-chave: diversificação, ciclagem biológica, fluxos de N, sustentabilidade, serviços ecossistêmicos, resiliência

1. Introduction

The spatial decoupling of agricultural and livestock production resulted from a highly specialized process of agriculture, under the bias of intensifying food production, especially in industrialized countries, in response to political and economic restrictions (Russelle et al., 2007; Hendrickson et al., 2008). The main consequences of the productivism race are environmental pollutions, fragmentation of “habitats” and diversity loss (Lemaire et al., 2014).

A classic example of specialized systems under conventional management is the rice-based cropping system, which occurs predominantly in an environment called lowlands on paddy fields in Rio Grande do Sul State. The conventional system of flooded rice cultivation occupies 1 million ha annually and is historically characterized by monocropping with winter fallow period, strongly linked to practices of intense and frequent soil tillage (SOSBAI, 2018).

The frequent soil tillage promotes soil disturbance, considered the main cause of soil degradation worldwide (Lal, 2015). The progressive disturbance in soil associated with a rapid increase in world demand for food, pressure on agricultural systems to operate more intensively and efficiently in order to reduce environmental impacts.

The simplification of agricultural systems outcomes an artificial ecosystem that requires constant human intervention, whereas the internal regulation of function is a product of plant biodiversity through flows of energy and nutrients, progressively lost in these conditions (Swift and Anderson, 1993). Biodiversity is known as a major determinant of community and ecosystem dynamics and functioning (Tilman, 2014). The increase in the level of diversity in the time and space of agricultural systems has been proposed as mechanisms to increase sustainability and resilience (Garrett et al., 2020).

Integrated crop-livestock system (ICLS) is an example of a diversified production system that are designed to exploit synergisms and emergent properties that result from interactions in the soil-plant-animal-atmosphere compartments on different spatiotemporal scales through the integration of crops and animals (Moraes et al. 2013).

Globally, ICLS are recognized as the most promising alternative for sustainable intensification to meet the growing demand for food (FAO, 2011) through

conservationist agricultural principles (Anghinoni et al., 2013). The scientific community recognizes that the ICLS is efficient in cycling nutrients and energy (Entz et al., 2005), more sustainable (Ryschawy et al., 2012), resilient (Lemaire et al., 2014; Peterson et al., 2020) and has more stability (Albuquerque et al., 2021).

The restoration of specialized agroecosystems in diversified systems with a high level of complexity in space and time allows the restoration of the dissociation of biogeochemical cycles, mainly through microbiological activity (Martins et al., 2017; Carlos et al., 2020; Sekaran et al., 2020).

Large herbivores play a dominant role in the cycling of nutrients in integrated systems, modifying the flows between the soil-plant-animal compartments, ingesting and consuming nutrients and profoundly modifies the dynamics of nutrients in the various compartments of the system (Anghinoni et al., 2011). The grazing promotes a continuous growth, consequently, prolonged demand for nutrients, thus being able to contribute to the mitigation of its losses, and this occurs due to the synergism between root growth and partial thinning of the plants leaves that stimulate regrowth (Moraes et al., 2014).

The magnitude of the animals' interference in nutrient cycling will depend on the distribution of excreta in the pasture, the area affected by the excreta and their nutrient content. The deposition of animal manure strongly influences the concentration of nutrients and in the microbial communities, consequently, enhance the availability of N and the decomposition of organic matter (McNaughton, 1992).

The recognition of the complexity of the interaction between the compartments and the transfer of fertilization between all cultures involved in a succession of a cultivation system continuum, a new fertilization philosophy emerges. 'System fertilization' is based on the principle of biology nutrient cycling between system phases to achieve efficient nutrient use and thus reduce the need for mineral nutrient input, avoid losses and maintain soil fertility long-term (Assmann et al., 2017).

Pasture fertilization can optimize fertilizer use, determined by nutrient (re)cycling through grazing, consequently improving soil fertility (Carvalho et al., 2010). The greater NUE in forage crops occurs because the ruminants export little amounts of nutrients via animal tissue, returning 70-95% of them from the plant to the soil, through excrement (Russelle, 1997). Studies in Brazil confirmed the viability of this concept in subsequence crop yield, as corn (Assmann et al., 2003; Sandini et al., 2011; Bortolli,

2016), soybean (Denardin et al., 2020a; Farias et al., 2020) and rice (Denardin et al., 2020b).

The great edaphoclimatic variability of Brazilian subtropic farms promotes a range of opportunities to compose integrated systems through diversification. In the rice scenario, there are cultivation alternatives, of varying intensity and diversity, which include commercial crops in the summer and forage species, both in the summer and in the winter, used in grazing.

Italian ryegrass is an adapted grass species recommended to compose ICLS in lowlands (Marchezan et al., 2005). N is the main factor that limits leaf development and the efficiency of the use of radiation on ryegrass (Viegas, 1998). The utilization of legume in mixed crops is a sustainable alternative to increase the N nutrition of ryegrass, decrease N fertilisers use (Walker et al., 1954; Simpson, 1965), improving N use efficiency (Corre-Hellou et al., 2006; Bedoussac and Justes, 2010).

The benefit for the grass associated in legume-grass mixtures comes from the lower competitive ability to capture N from soil by the legume (Soussana et al., 1989; Faurie, 1994), which increases the N availability for grasses (Vallis et al., 1967). In addition to the directly (Bethlenfalvay et al., 1991; Frey and Schiepp, 1992; Johansen and Jensen, 1996; Paynel et al., 2001), and indirectly N contribution (Hogh-Jensen and Schjoerring, 2001; Ledgard and Steele, 1992; Tobita et al., 1994).

The availability of N in the soil is generally an important limiting factor for the productivity of agricultural systems. Therefore, the use of fertilizers and legumes to supply the needs of N is one of the fundamental factors to meet the production of food to the growing demand of the human population (Angus, 2001).

The unpredictability in the supply of N according to the type of soil, management of the previous crop and climate, urges farmers to apply significantly higher amounts of N than the corresponding to maximum yield, which leads to greater costs and environmental pollution (Lemaire, Jeuffroy and Gastal, 2008). Even so, Lawlor, Lemaire and Gastal (2001) related that a large part of pastures does not receive N fertilizers in the world, which compromises the expression of the genetic potential of the crop.

The adjustment N inputs according to requirements of the crops corresponding to the target yield is a major challenge for farmers. A sufficient supply of N by fertilizers

can provide a sufficient amount of N for the plants to reach the potential growth allowed by the amount of energy intercepted by the crop (Lemaire, 1997).

The recommendations of N fertilization are based on the soil organic matter (SOM) content (CQFS RS/SC 2004; 2016), because N content in the soil has a high correlation with SOM (Mello et al., 1989; Schulten and Schnitzer, 1997). However, the determination of N fertilization management based on SOM analysis may be inaccurate, considering the great instability of N in soil and dependence on the C/N ratio of organic matter, this recommendation promotes an incomplete measurement of N status (Sartor, 2009).

Plant-based diagnostic of N status is a valuable tool for making decisions on the need to apply a supplemental amount of N (Lemaire, 1997). The Nitrogen Nutrition Index (NNI) is an example of plant-based diagnostic based on the concept of critical N concentration (N_c), defined as the minimum shoot N concentration necessary to obtain maximum growth rate (Ulrich, 1952). Values of N_c are high at the start of the growing period and decline during growth, in relation to dry matter accumulation ($DM, t DM ha^{-1}$) representing a dilution phenomenon (Farrugia et al., 2004).

We aim to evaluate the N status of Italian ryegrass under N levels and plant diversity in rice-based ICLS, in order to verify whether an increase in the diversity of plants in rice-based ICLS improves the Italian ryegrass NNI.

2. Material e Methods

2.1. Area description, experimental design and management of experimental protocol.

This study integrates an experiment site located at Corticeiras Farm, in Cristal district, Rio Grande do Sul State, Brazil (31°37'13" S, 52°35'20" W, 28 m). The region climate is classified as Cfa, characterized as a warm humid summer climate, according to Köppen (Kottek, Grieser, Beck, Rudolf, and Rubel, 2006). The mean annual temperature and precipitation is 18.3°C, and 1522 mm, respectively (30 -yr normal) (Matzenauer et al., 2011).

The experimental area has been cultivated with flooded rice over spring-summer followed by fallow period over winter-autumn from 1962 until March 2013. The soil is a Albaqualf (Soil Survey Staff, 2010), with sandy clay loam texture (24, 23 and 53% of clay, silt and sand, respectively).

The soil chemical analysis was performed in a diagnostic soil surface (0–10 cm soil layer) at the beginning of the experimental protocol. Soil chemical analysis presented 1.8 g kg⁻¹ of organic matter, pH of 5.5, 3.5 cmol dm⁻³ Ca, 2.3 cmol dm⁻³ Mg, 56% of base saturation, 3% of aluminium saturation, 10 mg dm⁻³ of available phosphorus, and 76 mg dm⁻³ of available potassium. Based in this soil chemical analysis and according to CQFS RS/SC (2004) was incorporated to the soil 4.5 Mg ha⁻¹ of limestone with 70% of total neutralization power to raise soil pH to 6.0 (0-20 cm soil layer).

After correction of soil pH, five rice-based ICLS with different spatio-temporal variability of plants species arrangement (Table 1) representing production models for different scenarios of paddy fields in RS were established in an 18-ha area. For the present study, four out of five systems were evaluated: 2, 3, 4 and 5, referred as S2, S3, S4 and S5, respectively.

The experimental design of this protocol consists in a randomized complete block with three replications. The experimental protocol consists in succession of summer crops used for grain production (except for grazed Sudan grass) and winter pasture used for grazing steers, arranged in different spatio-temporal variability of plants species.

Table 1. Rice-based systems description and spatio-temporal crops distribution at experimental protocol in Brazilian lowland.

	2017/18		2018/19		2019/20		2020/21	
	A/W	S/S	A/W	S/S	A/W	S/S	A/W	S/S
S1	F	Ri	F	Ri	F	Ri	F	Ri
S2	R ^a	Ri	R ^a	Ri	R ^a	Ri	R ^a	Ri
S3	R ^a	So	R ^a	Ri	R ^a	So	R ^a	Ri
S4	(R +P) ^a	Sg ^a	(R +P) ^a	So	(R +P) ^a	Co	(R +P) ^a	Ri
S5	(R+P+ B) ^a	Sf	(R +P+ B) ^a	Sf	(R +P+ B) ^a	Sf	(R +P+ B) ^a	Ri

F= fallow period; Ri= rice; So= soybean; Co= corn; R= ryegrass; P: Persian clover; B: birdsfoot trefoil; SF= sucession field; Sg= Sudan grass; S/S= summer/spring; A/W=autumn/winter.
^agrazed by bovine steers.

The experimental protocol is characterized to complete a cycle every four years, in which all cropping systems are cultivated with flooded rice in summer season. At first cycle (from 2013/14 to 2016/17), traditional criteria of crop fertilization were used, assigning a specific fertilization for each crop, according to certain response per yield expectations. Therefore, both crop and forage were fertilizer with respectives response per yield expectations (CQFS RS/SC 2004; 2016).

The present study comprises the last year of the second cycle of the experimental protocol (from 2017/18 to 2020/21), in which a new strategy of fertilization named system fertilization was established. System fertilization consists of applying the fertilizer in the phase of least nutrient extraction and high nutrient cycling capacity, that is, in the pasture phase (Assmann et al., 2017). In system fertilization, the fertilization level was stipulated based on greatest nutrients exportation crop and all fertilizer are applied in forage phase.

Grazing begins when the height of the pasture is approximately 20 cm, on average (around 1,500 kg of dry matter ha⁻¹). The continuous stocking method with

three tester steers and a variable number of "put-and-take" steers per experimental unit (paddock) were used to keep the target sward height at 15 cm in winter pasture (Mott and Lucas, 1952; Pontes et al., 2004) and 25 cm in summer pastures. The sward structure control was performed with 150 sward height measurements per paddock each 15 days, in random walk using a sward stick (Bircham, 1981; Barthram, 1986).

Animals remain in the paddock according to the date of sowing the crop in succession. The management of crops at experimental protocol (inoculation and/or seed treatment, herbicides, insecticides and fungicides) followed technical recommendations for pastures - CQFS RS/SC (2004; 2016); rice - SOSBAI (2016); maize, Sudan grass - FEPAGRO (2009) and soybean - EMBRAPA (2012).

In the winter-spring pasture growing season of 2020, a trial was conducted at experimental area. The pastures were sown on May 15, 2020, at the rate of 20, 6.5 and 8 kg ha⁻¹ of Italian ryegrass, persian clover and birdsfoot trefoil, respectively. The density remained fixed regardless of whether the systems were pure or mixed.

The experimental design was conducted as a split-plot randomized complete block design with three replications. Treatments were a factorial arrangement of four ICLS (main plots) and three N rates (subplots). The subplots occupied a total area of 30 m² per experimental unit (10 m² each rate) demarcated and excluded from grazing.

Three levels of N (0, 150 and 300 kg N ha⁻¹), subsequently referred as N0, N150 and N300, respectively, were surface-broadcasted on July 10. To avoid phosphorus and potassium deficiency, all treatments received phosphate and potassium fertilizer by surface broadcasted. On July 15 were applied 115 kg ha⁻¹ of P₂O₅ and on August 8, 90 kg ha⁻¹ of K₂O. Fertilizer sources used were urea (46% N), single superphosphate (18% P₂O₅) and potassium chloride (60% K₂O).

2.2. Soil analyses

Chemical soil analysis was performed in May 2020 in a diagnostical soil surface (0 – 10 cm, Table 2). All soil samples were submitted according to the methodology described by Tedesco et al. (1995).

Table 2. Soil analysis of the experimental protocol integrated crop-livestock systems in lowlands in May 2020 (0-10 cm layer).

	SOM ^a	pH ^b	P	K	Ca	Mg	CEC ^c	V ^d
	%		-----mg/dm ³ -----		-----cmol/dm ³ -----			%
S2	2.7	5.2	32.0	43.0	3.1	1.7	12.9	39.4
S3	2.2	4.9	29.3	62.0	2.6	1.5	13.7	30.5
S4	2.4	5.1	50.9	80.7	3.1	1.9	12.9	37.6
S5	3.0	5.3	55.9	88.0	3.6	2.3	13.0	46.8

^a Soil organic matter.

^b pH (H²O).

^c Cation exchange capacity.

^d Base saturation.

2.3. Plant analyses

The samples were collected in two dates, as the subplots referring to N0 did not yet presented the minimum of 1 t ha⁻¹ of mass required by the following methodology at first sampling. Therefore, in August and September, forage mass samples of N150 and N300; and N0, respectively, were collected at the soil level within a rectangle of 0.1 m², with three replications per rate. The rectangle was allocated at representative points of the sward height of each treatment, to estimate aboveground biomass and to evaluate N content.

Those samples were separated into botanical composition and the samples were dried in a forced air oven at 65 °C until reaching constant weight for mass and grasses:legume proportion measurements. After botanical composition separation, the plant analyzes were performed only on Italian ryegrass.

The determination of critical N (%N_c) was performed by calculating the critical nitrogen content in plants (%N_c) calculated for temperate pasture (C₃ species) according to Lemaire (1997) using the equation 1:

$$\%N_c = 4.8W^{-0.32}, \quad [1]$$

Where W is the total shoot biomass expressed in Mg dry matter (DM) ha⁻¹, N_c is the total N concentration in shoots expressed in % DM, and 4.8 and 0.32 are estimated parameters. The procedure for estimating the value of the nitrogen nutrition index (NNI) was based on NNI_{comp} index (3) proposed by Corre-Hellou (2005) for mixed species. The NNI_{comp} assumed the hypothesis of a perfect asymmetry in the

interaction between species (e.g., growth and N content of the component species not affected by its neighbour), which is equivalent to the situation of a taller very dominant species (Schwinning & Weiner, 1998), as Italian ryegrass in this study. The NNI comp is the ratio of the actual N, determined according to the Kjeldahl method (AOAC, 1995; method No. 984.13) and the %Nc previously calculated.

$$\text{NNIcomp} = \%N_i / \%N_c \quad [2]$$

According to Lemaire, Jeuffroy and Gastal (2008), NNI values close to 1 indicate that there is no N limitation; values above 1 are considered surplus, which characterizes a “luxury” consumption by the plant, and being well nourished, it stores and accumulates in the tissues the unused N for the development and production of DM and values < 1 indicates deficiency of N. The intensity of the deficiency can be estimated by the NNI value: a value of 0.5 indicates that the N available in the plant represents 50% of the critical level for culture.

2.4. Statistical analyses

Data were submitted to analysis of variance (ANOVA) considering a split-plot design, with ‘system’ the main plot and ‘N level’ the subplot. All variables achieved the ANOVA assumptions of normality (Shapiro-Wilk test, $P > 0.05$), variance homogeneity (Bartlett test, $P > 0.05$) and independence of residuals (visual analysis). We used a mixed model for data analyses (by *lme* function of package *nlme* in R software), considering the fixed effects of system, N level and its interaction, and random effect for block within system (error a). When differences were detected between the studied effects, means were compared by Tukey test ($P < 0.05$) using the packages *emmeans* and *multcomp*. All statistical analyses were performed using the R software (version 4.0.2).

3. Results

A statistical difference between levels (N0, N150 and N300) for all variables analyzed ($p < 0.0001$) was observed (Table 3). Although no differences in DM and N content between systems were observed, NNI was significantly different ($P = 0.0276$, Table 3). The highest NNI average was observed in S3 ($P = 0.94$), not differing from

S4 ($P = 0.88$) (Table 3). The lowest NNI values were observed in S2 and S5, with an average of 0.77 (Table 3). On the other hand, there was a statistical difference in all variables for N rates (Table 3).

DM didn't show interactions between systems and N levels ($P = 0.1111$) and difference between systems ($P = 0.1525$), with an average of 3.23 t ha⁻¹ (Table 3). There was a difference between N levels ($P < 0.0001$). A superiority was observed in the doses of 150 and 300 kg N ha⁻¹ (3.99 t ha⁻¹) and no statistical difference was detected between them (Table 3).

No difference between systems was found for N content ($P = 0.2476$), with an average of 27.7 g kg⁻¹ (Table 3). However, was observed a difference between N levels ($P < 0.0001$) (Table 3). The highest N content was observed at N300, the intermediate was N150 and NO was the lowest. As in DM, no interaction between systems and N levels was observed ($P = 0.1650$) (Table 3).

Table 3. Dry matter, nitrogen content (N) and nitrogen nutrient index (NNI) under three levels of N (kg N ha⁻¹) in different integrated crop-livestock systems in lowlands.

	N level			Average	P _s	P _N	P _{S*N}
Systems	0	150	300				
	Dry matter (t ha ⁻¹)						
2	1.51	3.68	4.04	3.08	0.152	<0.0001	0.1111
3	1.96	4.71	5.24	3.97			
4	1.56	4.19	4.70	3.48			
5	1.83	2.30	3.06	2.40			
Average	1.72 b	3.72 a	4.26 a				
	N (g kg ⁻¹)						
2	14.7	27.2	34.6	25.5	0.247	<0.0001	0.1650
3	16.8	33.7	35.8	28.8			
4	20.3	29.3	35.7	28.4			
5	19.8	30.6	33.7	28.0			
Average	17.9 c	30.2 b	34.9 a				
	NNI						
2	0.35	0.85	1.12	0.78 b	0.027	<0.0001	0.0595
3	0.43	1.14	1.26	0.94 a			
4	0.48	0.96	1.22	0.88 ab			
5	0.49	0.81	0.97	0.76 b			
Average	0.44 c	0.94 b	1.14 a				

Note. Means followed by lowercase letters in the columns and rows differ from each other by the Tukey test ($P < 0.05$).

The N content of Italian ryegrass forage mass, according to the different N levels, were exposed in the dilution curve proposed by Lemaire and Gastal (1997) (Figure 1).

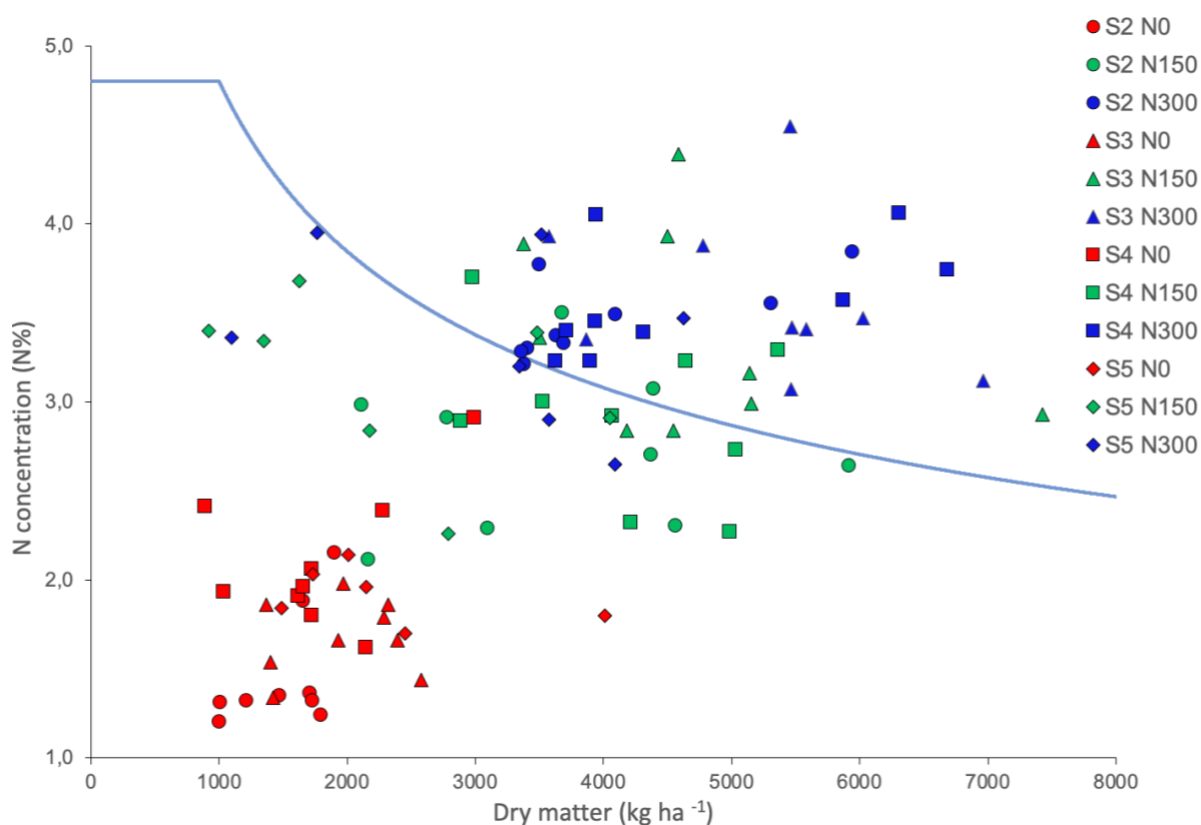


Figure 1. Italian ryegrass N concentration (N%) in relation to dry matter (kg ha⁻¹) for each N fertilization level in different integrated crop-livestock systems using the Lemaire and Gastal (1997) (blue line). S2) Italian ryegrass following rice; S3) Italian ryegrass following a rotation of rice and soybean; S4) Italian ryegrass and persian clover mixed following a rotation of rice, Sudan grass, soybean and corn and; S5) Italian ryegrass, persian clover and birdsfoot trefoil mixed following a 3-year rice succession field. N0) No N applied; N150) 150 kg N ha⁻¹ and N300) 300 kg N ha⁻¹.

A statistical difference between systems ($P = 0.0276$) was observed in NNI (Table 3). The highest NNI was observed in S3 ($P = 0.94$), not differing from S4 ($P = 0.88$) (Table 3). The lowest NNI values were observed in S2 and S5, with an average of 0.77. There was a difference between N levels ($P < 0.0001$) (Table 3). As well as in DM, the highest NNI was observed at N300 (1.14), intermediate at N150 (0.94) and lower at N0 (0.44). There was no interaction between systems and N levels ($P = 0.0595$). However, a great proximity with significance level ($\alpha = 0.05$) was detected.

4. Discussion

The NNI was positively affected by an increase in N fertilization level (Table 1). It is observed that the N concentration was below the critical curve in the treatments with N0, characterizing a N deficiency (Lemaire et al., 1997). The N150 level reached NNI values close to 1 in all systems (0.94), which indicates that N level of 150 kg N ha⁻¹ is closely from N critical concentration for Italian ryegrass independent on the ICLS spatial-temporal arrangement.

The N300 corresponds to double of N150 rate and presented 21% greater NNI (1.14) compared to N150 (0.94). Also, results showed that at N300, all systems did exceed the NNI excepts for S5. This result indicate that the nitrogen plant requirement was supplied (Lemaire and Gastal, 1997).

Although was found no statistical difference ($p > 0.05$) between the systems for DM and N content, S3 presented the greatest values at all levels applied. This can be attributed to the higher legume frequency during summer crop and its contribution through N mineralization of the soybean residue (predecessor crop).

The N concentration (N%) found in this study is in accordance with previous studies, in which the percentage of N% varied from 10 to 55 conducted with annual and perennial ryegrass, according to the supply of N and environmental conditions (Marino et al., 2004; Gislum; Boelt, 2009; Menezes, 2019).

Marino et al. (2004) calculated NNI for annual ryegrass and observed an increase in forage N% in response to increases in N rates, but a nonsignificant difference in forage yields were observed beyond N100. The authors found a NNI lower than 0.5 for 0 and 50 kg N ha⁻¹, close to 1.0 at 100 and 150 kg N ha⁻¹, and higher than 1.0 at 200 and 250 kg N ha⁻¹.

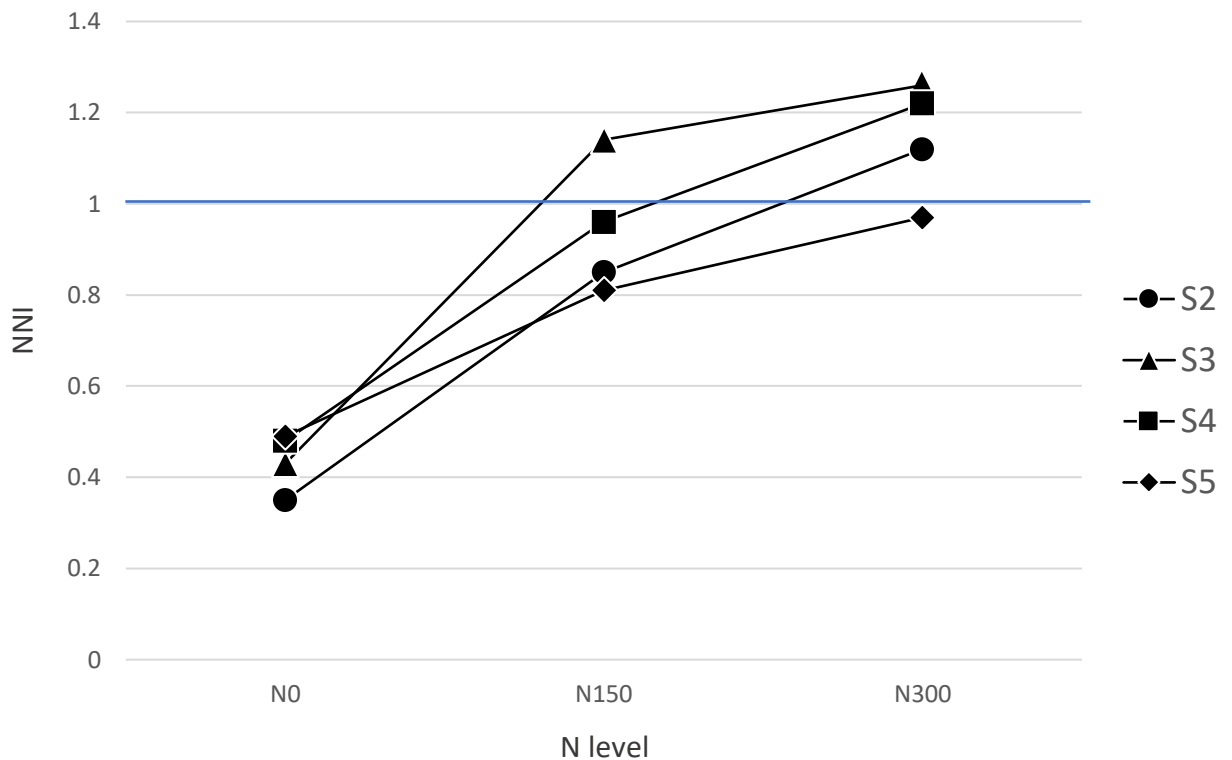


Figure 3. Comparison of NNI between systems for each N fertilization level during winter-spring growth of Italian ryegrass. S2) Italian ryegrass following rice; S3) Italian ryegrass following a rotation of rice and soybean; S4) Italian ryegrass and persian clover mixed following a rotation of rice, Sudan grass, soybean and corn and; S5) Italian ryegrass, persian clover and birdsfoot trefoil mixed following a 3-year rice succession field.

In the treatments without N (N0), systems with mixtures in winter (S4 and S5) showed higher values of NNI than pure systems (S2 and S3). Which suggests a greater contribution of N from legumes biological N fixation due to the greater efficiency under lower levels of inorganic N. This is in agreement with Cruz et al. (1991), which observed a positive effect of the presence of the legume on the N nutrition of the grass under N-limiting conditions. Whereas, when an ample N fertilizer was supplied, the positive effect of the legume disappeared.

Hoglund and Brock (1987) reported that the presence of inorganic N, promotes a reduction in N₂ fixation by reducing nitrogenase activity, due decreasing the formation of nodules and, sometimes, an increase in senescence of the nodules. In the grass-clover intercropped, enhance inorganic N also increases grass competition reducing legume participation and, consequently, reduce N₂ fixation per unit area (Whitehead, 1995).

The S2 presents the lower diversity level of the cultivated species and the highest intensity of rice cultivation, with no legume crop introduction. The rice has a high amount of residue and high C:N ratio (above 25 g g⁻¹) which causes a lower decomposition rate and N release since the microorganisms will immobilize the N temporarily and there will be no accumulation of mineral N, until the balance is reached (Manzoni et al., 2008). For this reason, the S2 showed low NNI at N0, since the N content from mineralization was low.

The S3 represents the faster rotation of grass-legumes summer crops and the higher frequency of legume summer crops. Although no legume-grass mixtures are present in winter forage of S3, the temporal biodiversity of the crop rotation can provide similar underground benefits such as spatial diversity, such as positive effects on soil C and N (McDaniel et al., 2014). Which can explain the change in the flows with the application of 150 and 300 kg⁻¹ N ha⁻¹ in the S3, which the pure Italian ryegrass presented the highest values.

The S3 was preceded by soybean, characterized by low residue C: N ratio. Residues containing less than 25 g N g⁻¹ DM promote a soil N contribution by mineralization, since the net immobilization of N decreases and the mineralization process is initiated (Manzoni et al., 2008).

Many authors studying nutrient cycling have quantified the N content from the soybean residue. Assmann et al. (2015) observed that soybean leaves through the residue of its shoot an amount of residual N on average 46 kg ha⁻¹, which are released gradually over the pasture cycle that will come in succession of ICLS with soybean-pasture rotations. Toomsan et al. (1995) found that soybean contributed 15 kg N ha⁻¹ to the total benefit of residual N. Heichel and Barnes (1984) found that an estimated value of 24 kg N ha⁻¹ returned to the soil, when 90% of N is biologically fixed.

The S4 presented the greatest diversity of cultivated plants, which indicates a greater introduction of matter and energy into the system. The NNI of S4 reached the critical value of NNI at the doses of 150 and 300 kg⁻¹ N ha⁻¹, which can be attributed to the greater diversity of agricultural crops, which according to McDaniel et al. (2014) allows residues with different qualities to directly affect soil C and N stocks and microbial diversity and activity, ultimately impacting the entire nutrient cycling process.

The S5 has the greatest diversity of winter species cultivated and the highest animal frequency. The highest SOM (3%) indicates that there is a greater contribution of C

and N driven by microorganisms through the processes of N mineralization of plant and animal waste, which explains the greater NNI at N0. In addition to the contribution of the mineralization of crop residues, the duration of the grazing period also contributes to the amount of N, mainly due to the great influence on the microbial population and concentration of nutrients, causing an increase in the availability of N and the speed of decomposition of organic matter (Mcnaughton, 1985).

In this sense, the S5 has the greater grazing period because the animals remain in the area uninterruptedly, with no cultivation of summer crops. In the 2014/2015 cropping system, the grazing period reached 336 days and was a production of 1.074 kg DM of manure ha⁻¹, covering up to 276 m² ha⁻¹. While the shortest grazing periods (S2) corresponded to 54 days, producing less than 200 kg DM of manure ha⁻¹. N was the nutrient with the highest concentration in feces, reaching 35, 9, 5 and 4 kg⁻¹ N ha⁻¹ in S5, S4, S3 and S2 (Borin-unpublished date).

Conversely, when 150 and 300 kg N ha⁻¹ were applied, S5 showed the lowest NNI, corresponding to 0.81 and 0.97 respectively, although it does not differ from S2. The lowest values in S5 can be attributed to the absence of added crops in the summer, which allowed the establishment and development of *Poa annua* and generate an infestation in the following season.

Despite the greater diversity of cultivated winter species in S5, no summer species introduced represents a low addition of residues and consequently of matter and energy for the processes linked to microbiological activity and soil functional.

The insertion of summer legume crops had a positive influence on the processes of re-coupling the C and N cycles in agroecosystems in order to allow a better use of the applied fertilizers. In this work, the ICLS involving two summer crops rotation and one winter forage crop obtained the same NNI as rotations with four summer crops rotation and two winter forage crops mixtures (grass-legume mixed).

5. Conclusion

The increase in the spatio-temporal plant diversification in ICLS promotes a greater NNI. The higher frequency of leguminous crops in the summer proved to be as effective as the increase in the level of diversification of cultivated species to improve the NNI.

In these field conditions, there is an indication that the Nc levels corresponding to the Nc for Italian ryegrass are between 150 and 300 kg⁻¹ N ha⁻¹.

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CHAPTER III

FINAL CONSIDERATION

The process of investigating the processes that occur belowground and their interaction with the compartments aboveground is composed of several steps that, through small explanations, allow the advancement of knowledge and promote new inquiries. In this sense, science is unstatic and are constantly confronted with new findings that allow new questions. It is the role of science to produce content and it is essential that this content reach society, since research is the cause and consequence of social demands and aspirations.

In agriculture, N issue is increasingly studied and its extremely important because it involves all areas that comprise environmental, economic, agronomic and social sustainability. The internal regulation of nutrient cycling must be handled with the knowledge of the importance of providing the system with resources that allow its self-regulation. This does not mean that the use of inputs is unnecessary in these systems, but it means that if the ecological functioning of the systems is understood, these systems will have better use of inputs, through greater use efficiency, especially N fertilizers, which the complexity dynamics makes it extremely difficult to measure. Lower losses mean lower costs, and' more than that, in a holistic view, ensuring internal regulation.

To enable the practical application of N diagnosis in the field, it is necessary to use methods less timeconsuming to consume and simple. New methods have been developed for this purpose. Among them, a great correlation was found for N of upper leaves (N_{up}), a new method based on the principle that the N concentration in leaves at the top of the canopy remains constant during growth. The relationship between N_{up} and NNI could also be valuable in remote sensing methodologies where optical properties of the canopy are more dependant on properties related to N concentration at the top of the canopy than the entire above-ground herbage.

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