Study of macronutrient dynamics and biomass production of soybean (Glycine max (L.) Merr.) in no-till integrated crop-livestock systems under two types of fertilisation in southern Brazil

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MASTER THESIS PRESENTED FOR THE OBTAINING OF A DEGREE IN BIOENGINEERING IN AGRICULTURAL SCIENCES

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(CO)-PROMOTER(S): PR. JÉRÔME BINDELLE AND PR. PAULO CÉSAR DE FACCIO CARVALHO

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Abstract

As a result of rising food demand and evolving production technologies, modern agriculture is now characterised by standardised and efficient monoculture systems. This type of agricultural production is common in rural areas around the world, but is beginning to show signs of saturation due to its high demand for energy and natural resources. Dependence on chemical fertilisers has a significant impact on soil fertility, making farming systems increasingly vulnerable. Integrating livestock into farming systems has been shown to improve system resilience. In addition, a new approach to fertilisation is emerging, known as systemic fertilisation. This technique is based on the conceptual framework that fertilisers should be applied during the phase of the system when nutrient extraction is lowest and nutrient recycling capacity is highest. To evaluate the combination of these two promising techniques, this work compared the macronutrient dynamics and biomass production of soybean (*Glycine max* (L.) Merr.) in integrated cropping systems with a cropping system subjected to two types of fertilisation in the Rio Grande do Sul region of southern Brazil. The conventional cropping system consisted of a soybean crop followed by an non-grazed Italian ryegrass (*Lolium multiflorum* Lam.) cover crop. The ICLS system consisted of a rotation of soybean and Italian ryegrass grazed by sheep in winter. In the conventional fertilisation strategy, phosphorus and potassium were applied when the soya was sown and nitrogen when the Italian ryegrass was planted. In the fertilisation system used, all nutrients were applied at the time of Italian ryegrass establishment. The results indicate that the integration of ICLS and systemic fertilisation improved the availability of nutrients in the soil. This allowed the plant to assimilate these elements more efficiently during its vegetative growth cycle, leading to an increase in biomass production compared to ungrazed systems. Favourable soil nutrient conditions led to an increase in the photosynthetic parameters of ICLS soybean plants, which declined more slowly at the end of the cycle. However, no difference in yield was observed between the systems. This could be explained by methodological limitations, differences in sampling or the possible influence of abiotic factors. In conclusion, this study has shown that ICLS combined with systemic fertilisation is a potential means of increasing food production and improving the sustainability and productivity of agro-ecosystems. However, in view of the results of the present study, there are a number of avenues for further research, such as further investigation of soil-plant interactions at different horizons or the development of predictive models in different soil and climate contexts.

Keywords: modern agriculture, monoculture, soil fertility, integrated crop-livestock systems, soybean, italian ryegrass, sustainability, southern Brazil

Résumé

En raison de l'augmentation de la demande alimentaire et de l'évolution des technologies de production, l'agriculture moderne se caractérise aujourd'hui par des systèmes de monoculture standardisés et efficaces. Ce type de production agricole est courant dans les zones rurales du monde entier, mais commence à montrer des signes de saturation en raison de sa forte demande en énergie et en ressources naturelles. La dépendance à l'égard des engrais chimiques a un impact significatif sur la fertilité des sols, ce qui rend les systèmes agricoles de plus en plus vulnérables. Il a été démontré que l'intégration de l'élevage dans les systèmes agricoles améliorait la résilience de ces derniers. En outre, une nouvelle approche de fertilisation, connue sous le nom de fertilisation systémique, est en train d'émerger. Cette technique est basée sur le cadre conceptuel selon lequel les engrais doivent être appliqués pendant la phase du système où l'extraction des éléments nutritifs est plus faible et où la capacité de recyclage des éléments nutritifs est plus élevée. Pour évaluer la combinaison de ces deux techniques prometteuses, ce travail a comparé la dynamique des macronutriments et la production de biomasse du soja (*Glycine max* (L.) Merr.) dans un système intégré culture-élevage avec un système de culture conventionnel soumis à deux types de fertilisation dans la région du Rio Grande do Sul, dans le sud du Brésil. Le système de culture conventionnel consistait en une culture de soja suivie d'une culture de couverture de ray-grass italien non pâturée. L'ICLS consistait en une rotation de soja et de ray-grass italien (*Lolium multiflorum* Lam.) pâturé par des moutons en hiver. Dans la stratégie de fertilisation conventionnelle, le phosphore et le potassium ont été appliqués au moment de l'ensemencement du soja et l'azote au moment de la plantation du ray-grass italien. Dans le système de fertilisation utilisé, tous les nutriments ont été appliqués au moment de l'implantation du ray-grass italien. Les résultats indiquent que l'intégration de l'ICLS et de la fertilisation systémique a amélioré la disponibilité des nutriments dans le sol. Cela a permis à la plante d'assimiler ces éléments plus efficacement pendant son cycle de croissance végétative, ce qui a entraîné une augmentation de la production de biomasse par rapport aux systèmes non pâturés. Les conditions nutritives favorables du sol ont entraîné une augmentation des paramètres photosynthétiques des plants de soja ICLS, qui ont diminué plus lentement à la fin du cycle. Cependant, aucune différence de rendement n'a été observée entre les systèmes. Cela pourrait s'expliquer par des limitations méthodologiques, des différences d'échantillonnage ou l'influence possible de facteurs abiotiques. En conclusion, cette étude a montré que l'ICLS combiné à la fertilisation systémique est un moyen potentiel d'augmenter la production alimentaire et d'améliorer la durabilité et la productivité des agro-écosystèmes. Cependant, au vu des résultats de la présente étude, il existe un certain nombre de pistes de recherche, telles que l'étude plus approfondie des interactions sol-plante à différents horizons ou le développement de modèles prédictifs dans différents contextes pédoclimatiques.

Mots-clés: agriculture moderne, monoculture, fertilité des sols, systèmes intégrés culture-élevage, soja, ray-grass italien, durabilité, sud du Brésil

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Chapter 1

Introduction

The growth in global demand for food and the development of new technologies in agricultural production have contributed to the emergence of standardised and efficient monoculture systems. These modern systems are distinguished by the extensive utilisation of agrochemicals, irrigation, the expansion of agricultural frontiers, the mechanisation of soil management, and the intensification, independence and segregation of forestry, livestock and agricultural activities. Despite its prevalence on rural farms globally, this model is exhibiting indications of saturation due to its substantial energy and natural resource requirements (Balbino et al., 2011).

Modern agriculture relies heavily on chemical fertilisers to maintain crop productivity. This dependence has significant negative impacts, including water pollution, soil degradation and high economic costs for farmers (Machado et al., 2011). Furthermore, chemical fertilisers can reduce soil biodiversity and disrupt natural nutrient cycles, making farming systems more vulnerable to environmental disturbances.

This situation poses a major challenge to global food security, defined as the availability of a sufficient quantity of food of acceptable quality to ensure constant nutritional well-being, where all physiological needs are met. Food security rests on four fundamental pillars: (i) food stability; (ii) food availability; (iii) food access; and (iv) food utilisation. The mere presence of food does not guarantee an adequate diet in terms of calories and nutrients. Moreover, the consumption of high quality, often more expensive, foods can exacerbate global nutritional problems, particularly for low-income populations. With the world's population expected to reach 9.7 billion by 2050 and 10.9 billion by 2100, food security faces considerable challenges due to growing food consumption. Difficulties in accessing quality food, particularly for small farmers and landless families in low- and middle-income countries, exacerbate these problems (Sekaran et al., 2021).

In this context, soybean (*Glycine max* (L.) Merr.), originally from China, stands out as a strategic crop on a global scale. It is one of the main sources of vegetable oil and protein for animal feed. With a protein content of 40 to 42%, the highest among food crops, and an oil content of 18 to 22%, soybean plays a crucial role in improving global food security. Because of its nutritional value and versatility, soybean has become essential in meeting growing global food demand. Countries such as Brazil, the United States and Argentina dominate global soybean production, accounting for over 92% of the world's supply (Pagano & Miransari, 2016). Brazil, in particular, has become a major player, exporting a third of the world's supply using just 6% of its arable land. The sustainability of soybean production, particularly in tropi-

cal regions, is increasingly dependent on advances in fertilisation methods, including the use of biological nitrogen fixation, an environmentally friendly alternative to chemical fertilisers (Rodríguez-Navarro et al., 2011).

Faced with these challenges, integrated crop-livestock systems (ICLS) are proposed as a potential solution for improving soil fertility while reducing dependence on chemical fertilisers. These systems combine crop and livestock production on the same plot of land, making more efficient use of resources and creating synergies between the different agricultural components. ICLS can improve soil structure and fertility through the addition of organic matter and integrated nutrient management, while diversifying income sources for farmers. Integrating livestock into farming systems has been shown to improve land-use efficiency. In addition, the introduction of a less risky activity into specialised cropping systems makes it possible to better withstand climatic hazards and price volatility, thereby strengthening the economic resilience of the system (Carvalho et al., 2021). According to Vilela et al. (2011), these systems have the potential to transform current agricultural practices and offer viable solutions to environmental and economic challenges.

The aim of this study is to investigate the impact of integrated systems and different type of fertilisation on the dynamics of macronutrients and the biomass production of soybean crop over time, contributing to the development of more sustainable and efficient agricultural practices in Brazil and beyond.

Chapter 2

State of the art

I ICLS

I.1 ICLS definition

An Integrated Crop-Livestock System (ICLS) is a farming approach that combines the production of crops and livestock in a mutually beneficial way (Carvalho et al., 2021). It is defined by the rotation, consortium and succession of livestock and culture in the same place (Silva et al., 2022). It aims to create a more diverse and sustainable agricultural system by integrating grazing livestock into crop landscapes. It represents a shift from specialized to multifunctional landscapes, promoting biodiversity and various ecosystem services. Most of the time, pastures follow summer crops and can be sown alone or in combination, producing during a period when pasture is scarce. The unconsumed parts of the pasture, such as stems and old leaves, serve as cover for the subsequent crop, helping to suppress weeds and conserve soil moisture.

Indeed, ICLS offer a number of significant advantages. Firstly, they improve the economic performance of farms. Indeed, these systems also offer greater income stability by diversifying production sources, which reduces economic risks. The implementation of specialised farming practices has the potential to reduce the overall income of a production system by up to 75%. In comparison, the integrated system is observed to experience a loss of only 10% under the same climate change scenario (Seo, 2010). In addition, they contribute to a reduction in the use of chemical inputs; for example, integrating sheep into vineyards has reduced mowing costs and the use of herbicides (Sekaran et al., 2021). Those systems also restore the biogeochemical cycles (de Faccio Carvalho et al., 2010). Indeed, reintroducing livestock to crop areas can reconnect decoupled nutrient cycles, enhancing ecosystem functioning. Moreover, the integration promotes multiple ecosystem services, such as biodiversity, climate regulation and food production (*Figure 1*). Integrated crop-livestock systems can increase biodiversity via the attributes of organic matter provided by pastures (de Faccio Carvalho et al., 2010). The resulting diversity of flora and fauna, in conjunction with the soil's microbial and faunal communities, exert a modifying influence on the soil and its physico-chemical properties. The integrated systems can also better withstand weather and market fluctuations, providing biophysical and socioeconomic stability.

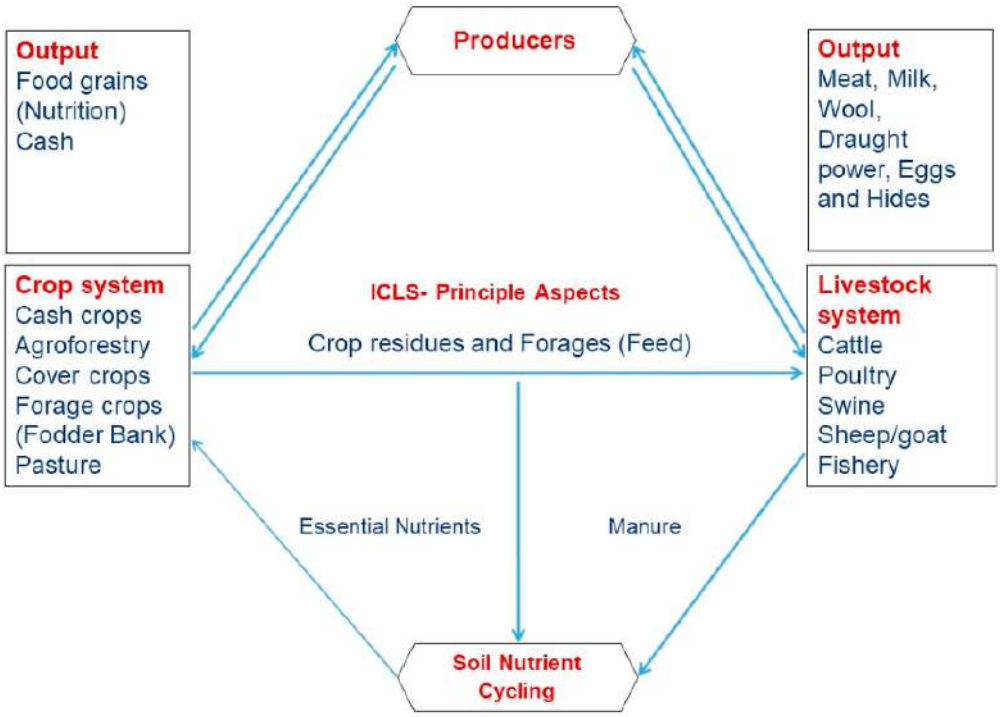


Figure 1: Simplified ICLS interaction scheme (Sekaran et al., 2021).

The primary concern among farmers contemplating the implementation of ICLS at the plot level is the potential impact of ruminant hooves on soil compaction and subsequent crop yield. However, the existing literature is inconclusive, with studies reporting potential reductions, no effects, or even increases in crop yields resulting from the integration of animals into agro-ecosystems (Carvalho et al., 2021).

Furthermore, there are some social and cultural barriers which are holding back the large-scale development of these systems. Those barriers are due to the complexity of managing multiple interactions between crops, livestock, and grassland. These systems require a great deal of agricultural knowledge and commitment, as the livestock need to be constantly monitored by the farmers (Peterson et al., 2020). Government assistance and particular regulations that safeguard smallholder subsistence farmers are essential for smallholder farmers to succeed in this endeavour and address the obstacles posed by ICLS (Carvalho et al., 2021). Government policies and support should give farmers access to the following: (i) capital support in the form of microcredit, in-kind loans for livestock, and government subsidies; (ii) increased investment in research into technologies and management related to livestock production; (iii) markets and support services to help them sell their agricultural products; (iv) education about the resilience of the ICLS; and (v) agricultural insurance (Sekaran et al., 2021).

Producers who have successfully transitioned to ICLS report that while there are challenges, the benefits of such systems can be significant when managed properly (Carvalho et al., 2021). Indeed, the use of appropriate grazing intensity throughout the pasture phase, a crucial management variable impacting sward structure, is in reality an important component. Grazing intensity affects animal performance by altering the structure of the grassland, which has an impact on grass intake, as well as potential effects on crop development due to physical alterations to the surface soil or nutrient recycling (Oliveira et al., 2014). The aim of the ICLS

is to strengthen the resilience of agricultural systems to the effects of climate change. This is achieved by employing buffering mechanisms at both the field and farm-level, which encompass a range of strategies including improved crop productivity, nutrient cycling, economic risk mitigation and livelihood diversification (Sekaran et al., 2021).

I.2 Origin of ICLS

The ICLS is part of a more global system called : Crop-livestock-forest integration system (ICLFS). This encompasses diversified production systems for the production of food, fiber, energy, and forest products, both woody and non-woody, of animal or plant origin, in order to optimize the biological cycles of plants and animals, as well as the respective inputs and their residues (Machado et al., 2011). In practice, there are four types of integrated production system that can be easily identified, each made up of a large number of arrangements and models derived from different edaphoclimatic, economic, social and cultural conditions. There are the crop-livestock integration system (agropastoral or ICLS), the livestock-forestry integration system (silvopastoral), the crop-forestry integration system (silvicultural) and the crop-livestock-forestry integration system (agrosilvopastoral or ICLFS) (Wruck et al., 2015).

Although ICLFS systems are considered innovative, in Europe, various forms of plantations combining annual and perennial crops or fruit crops and forest trees have been known since the Middle Ages. Systems integrating fruit trees and animal production date back to the 16th century, and their near-disappearance is attributed to the mechanization and intensification of agricultural systems, as well as the difficulty of manual fruit harvesting and administrative issues. Several Roman writers from the 1st century AD, including Caius Plinius, author of the encyclopedia *Natural History*, and Lucius Junius Moderatus, known as Columella, referred to integrated systems between trees (walnuts and olives) and pastures (Machado et al., 2011).

The tradition of mixing agriculture, cattle, and forestry—which was initially tailored to tropical and subtropical conditions—was brought to Brazil by European immigration. For instance, grazing and flooded rice farming were combined in Rio Grande do Sul. The most notable example of intercropping in the tropics is seen in the practices of small farmers. Nevertheless, despite recent scientific advancements, Brazil's use of integrated systems has remained low throughout time.

I.3 Brazilian context

However, in the current Brazilian context, these integrated systems make perfect sense. This technique makes it possible to optimise the space used by combining livestock and crops, avoiding the need to clear new areas of native forest. ICLS is the most widely used ICLFS strategy in Brazil, particularly in the South region, where crop and livestock farming are strongly present. It is particularly well accepted by soybean growers. Great expectations exist around ICLS as an alternative for achieving productivity while conserving resources (Wruck et al., 2015). Those systems are a more efficient land-use alternative, and this is reinforced by the low economic return from extensive livestock farming and the vast expanse of degraded pasture (Vilela et al., 2011).

Great expectations exist around ICLS as an alternative for achieving productivity while conserving resources (Wruck et al., 2015). ICLS are becoming increasingly important due to the difficulties faced by livestock farmers in investing in pasture rehabilitation and by farmers in restoring the productive potential of crops, mainly because of problems linked to the reduction in soil organic matter and the presence of insects, diseases and nematodes (Machado et al., 2011).

II Soybean

In the southern region of Brazil, approximately 6.4 million hectares are cultivated on an annual basis with soybean (*Glycine* max (L.) Merr.), maize (Zea mays L.) and rice (Oryza sativa L.). In comparison to continuous cropping, recent studies have demonstrated that the integration of pasture and livestock into a cereal crop rotation enhances the quantity and quality of soil organic matter. The present study focuses on the crop of soybeans, the characteristics of which are described in the following section.

II.1 Growth stages

The soybean plant's life cycle is separated into two stages: vegetative and reproductive. When the seed takes in water to initiate the germination process, the vegetative phase starts. When the first flower buds form in determinate soybean types, or the first raceme appears in indeterminate soybean varieties, the vegetative phase ends and the reproductive phase begins. Harvest marks the conclusion of the reproductive phase (Nleya, 2019). Legumes, including common beans such as soybean, possess the capacity to form symbiotic relationships with microorganisms, such as the genus Rhizobium, which enables them to fix atmospheric nitrogen (N2) and transform it into proteins (Reichardt & Timm, 2020).

Stage Number	Average Time Between Stages	Title	Description
Vegetative Stages	**# days**		
VE	Plt to VE 10d	Emergence	Cotyledons above soil surface.
VC	VE to VC 5d	Cotyledon	Unifoliate leaves unfold so leaf edges are not touching.
V1	VC to V1 5d	1st trifoliate	First trifoliate fully emerged and opened.
V2	V1 to V2 5d	2nd trifoliate	Plants have three nodes with two trifoliates unfolded.
V3	V2 to V3 5d	3rd trifoliate	Plants have four nodes with three trifoliates fully unfolded.
V4	V3 to V4 5d		
V5	V4 to V5 5d		
V6	V5 to V6 5d		
V(n)	V6 to Vn 3d	nth trifoliate	N= the number for the last fully developed trifoliate leaf.
Reproductive Stages	**Average Time Between Stages**	**Title**	**Description**
	# days		
R1	R1 to R2 3d	Beginning flowering	One open flower at any node on the main stem.
R2	R2 to R3 10d	Full bloom	Open flower at one of the two top nodes on the main stem with a fully developed leaf.
R3	R3 to R4 9d	Beginning pod	A pod on one of the four upper nodes in 3/16 inch (0.5 cm) long.
R4	R4 to R5 9d	Full pod	A pod ¾ inch (2 cm) long at one of the four uppermost nodes on the main stem
R5	R5 to R6 15d	Beginning seed	Seed is 1/8 inch (3 mm) long in one of the four uppermost nodes on the main stem.
R6	R6 to R7 18d	Full seed	A pod containing a green seed that fills the pod cavity at one of the four uppermost nodes on the main stem.
R7	R7 to R8 9d	Beginning maturity	One normal pod on the main stem has reached the mature pod color (tan or brown).
R8		Full maturity	95% of pods have reached mature pod color. Five to 10 days of dry weather are required to dry the seed to < 15% moisture.

Figure 2: Soybean plant growth stage and time invervals (Nleya, 2019).

The 3 key stages in this study are R1, R5.5 and R8.

R1 : Beginning bloom

Every node on the main stem has at least one open flower at this point. At this stage, soybean plants are 38–46 cm tall.

R5-R6 : Seed development

Plants are between 76 and 109 cm tall at this point. At one of the four upper nodes on the main stem, the plant bears pods with seeds that are at least 3 mm long. Rapid seed growth and the plant's internal redistribution of nutrients and dry weight are traits of the R5 stage. At the start of seed development, root growth slows down. At the end of this phase, the soybean plant has reached its maximum height, number of nodes, and leaf area. The rates of nitrogen fixation peak and then rapidly decline. Seeds acquire mass quickly. The process of transferring to the seed commences at the halfway point between R5 and R6, when dry weight and nutrient buildup in the leaves, stems, and petiole peak. The R5.5 sub-stage is the end of the critical period for reductions in yield potential (Nleya, 2019). This growth stage occurs approximately 7 days after R5 and marks a phase where node development has ceased and the number of seeds has been determined. The R6 stage begins when the seeds fill the pod cavity on at least one of the four upper nodes of the main stem of a plant. The average weight of the pods and the dry matter of the whole plant are maximum at R6 stage (Marques Pires et al., 2023). The growth rates of the seeds start to slow down and it's just after this stage that the leaf yellowing begins (Nleya, 2019).

R8 : Full maturity

At this stage, 95% of pods have reached their mature aspect (tane color). Five to ten days of drying weather are required after R8 before the soybeans have less than 15 percent moisture (Fehr & Caviness, 1977).

To achieve high yield potential, soybeans must maintain high rates of photosynthesis and accumulate large quantities of nitrogen in the seeds. Nitrogen exists in the leaves mainly in the form of ribulose bisphosphate carboxylase/oxygenase, and there is generally a close relationship between nitrogen per unit leaf area and photosynthesis. Therefore, the crop must have a canopy that allows complete light interception and sufficient nitrogen storage in the leaves to maintain a photosynthetic apparatus that is not limited by nitrogen, thus converting incoming radiation into new biomass and ultimately grain yield (Salvagiotti et al., 2008).

The yield response of soybean to nitrogen fertiliser application depends on the yield potential of the production environment and any abiotic or biotic constraints that reduce crop growth and the associated nitrogen demand. When such constraints exist, developing rhizobia strains capable of fixing N2 under stress conditions appears to be the most feasible way to ensure the necessary nitrogen supply (Alves et al., 2003). Some data suggest that techniques for providing additional nitrogen during grain filling without decreasing nodule activity are the most likely way to achieve a yield response to nitrogen fertilisation. Promising options include the deep placement of slow-release fertiliser below the nodulation zone or nitrogen application during the reproductive stages.

Detailed measurements of the uptake efficiency of applied N at different development stages and for various N application methods are crucial for understanding the reasons behind the response or lack of response to fertiliser N (Salvagiotti et al., 2008).

II.2 Photosynthesis

Photosynthesis phenomenon is providing the essential physiological basis for plant growth and biomass production. It represents the anabolic process through which plants convert solar energy into stable chemical energy in carbohydrates. Leaves, among all the photosynthetic organs are the most significant one for higher plants (Zhou & Yang, 2023). The majority of soybean crop biomass is derived from photosynthetic assimilates, with this figure exceeding 90% (Makino, 2011).

II.3 NPK in Soybean

Nitrogen is one of the main nutrients for soybean crops. It is a structural component of chlorophyll molecules and enzymes, which helps regulate the physiological processes of soybean cultivation (Bagale, 2021). Soybean crops require 80 kg of nitrogen to produce 1000 kg of seeds. Thus, efficient management of nitrogen fertilisers is important for achieving good yield and protein content in the soil. High concentrations of nitrogen fertiliser suppress nodulation and nitrogen fixation in soybean plants, leading to low grain yield (Pedrozo et al., 2018).

Then, phosphorus fertilisation is the major mineral nutrient yield determinant among legume crops (Chaudhary et al., 2008). Indeed, phosphorus is essential for the formation and functioning of root nodules in legumes, where biological nitrogen fixation (BNF) occurs. P is also crucial for cellular energy and energy transfer processes, which directly influence nitrogen absorption. A study by Sa & Israel (1991) showed that phosphorus deficiency reduces nodule

activity and nitrogen fixation efficiency in soybeans, decreasing overall nitrogen absorption by the plant (Chaudhary et al., 2008). Phosphorus is relatively immobile in the soil, so plants can exhibit deficiency symptoms if they are located too far from the phosphorus application zones. It reacts with other nutrients such as aluminum, calcium, and iron to form orthophosphate. If the phosphorus reacts, it is no longer available to the plant. A soybean plant requires 25 kg of phosphorus in the form of P2O5 to produce one ton of soybean seeds (Bagale, 2021).

Finally, potassium is a key element in several physiological processes, including protein synthesis, cell turgidity and nutrient transport within the plant. Potassium deficiency can limit the plant's ability to transport and assimilate nitrogen. Good potassium availability improves nitrogen use efficiency, which can increase nitrogen concentration in plant tissues (Tian et al., 2022). Moreover, this mobile nutrient plays an indispensable role in enzyme activation during nodulation (Bagale, 2021).

Therefore, combined fertilisation with phosphorus and potassium can have synergistic effects. Phosphorus enhances nitrogen absorption by increasing the number and activity of root nodules, while potassium aids in the mobilisation and efficient use of this absorbed nitrogen. Consequently, the application of P and K at the time of soybean planting can increase nitrogen availability and its absorption by the plants, reflected in higher nitrogen concentrations in plant tissues and in mature soybean pods (Chaudhary et al., 2008; Tian et al., 2022).

Soil organic matter (total and its fractions) is a key indicator of soil quality under different soil management regimes (Carvalho et al., 2018). Soil microbial attributes are also indicators of short-term changes in soil quality due to the role of micro-organisms in the synthesis and release of extracellular enzymes(Souza et al., 2014). Those are fundamental for the decomposition of plant residues and the mineralisation of nutrients (Babujia et al., 2010).

III Soil in ICLS

If only grazing residues are considered, it is expected that lower carbon stocks will be observed in grazed areas, as the lower the management height of the grassland, the lower the amount of residues, resulting in lower carbon accumulation. However, it is common for carbon stocks to follow a similar trend to that observed for total forage accumulation (*Figure 3*) (Carvalho et al., 2018). Grazing animals act as catalysts in the soil-plant-atmosphere continuum, modifying the rates and fluxes of processes and recycling organic matter. Moreover, grazing intensity affects nitrogen flows in the this continuum (Assmann et al., 2015).

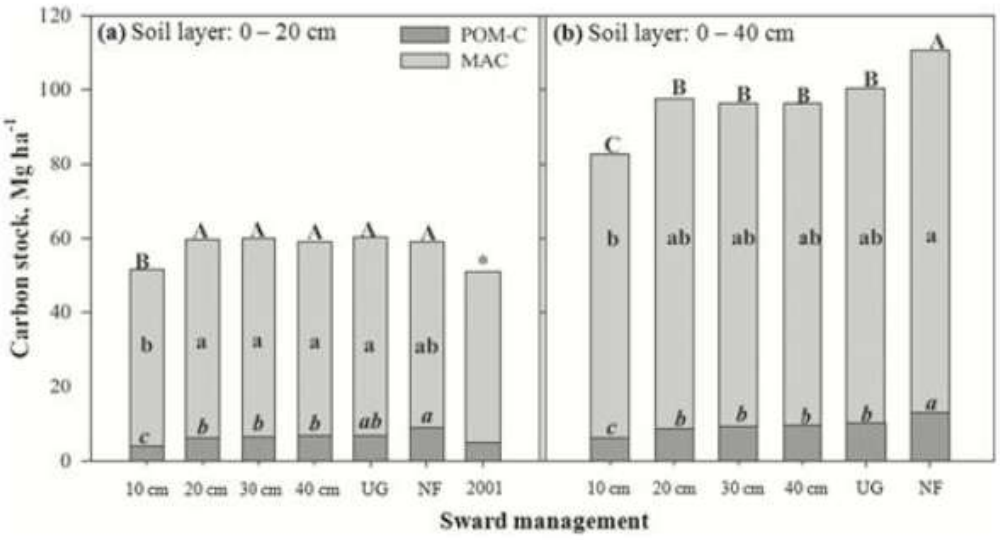


Figure 3: Total carbon, particulate organic matter carbon (POM-C), and mineral associated carbon (MAC) in 0 to 20 cm (a) and 0 to 40 cm (b) soil layers in an ICLS managed at different grazed cover crop heights (10 to 40 cm) or non-grazed (UG) in southern Brazil. NF = native forest. Different letters according to Tukey test ($P < 0.05$). (Carvalho et al., 2018).

IV Systemic fertilisation

Awareness of the nutritional requirements of plants and the utilisation of inorganic fertilisers facilitate an enhancement in crop yield. On an annual basis, the demand for fertiliser is increasing by 1.4, 2.2 and 2.6% (FAO, 2015). Therefore, there is a growing concern about the limited availability of mined fertilisers and the potential for contamination of water bodies (Farias et al., 2020).

Current fertiliser recommendations aim to meet crop needs and increase soil nutrient levels beyond critical thresholds (Sartor et al., 2018). However, conserving nutrients is key to agroecosystem efficiency. Thus, a new fertilisation approach has emerged: systemic fertilisation, based on the conceptual framework that fertiliser should be applied during the phase of the system with lower nutrient extraction and higher nutrient cycling capacity. Sheep grazing in winter can only export 5% of potassium and phosphorus in ICLS (Alves et al., 2019). An application of these nutrients in winter would maximise the total production of the system. This new approach considers all the benefits of well-managed grazing during the grazing phase, including reduced nutrient extraction by livestock and accelerated nutrient cycling returned to the soil through excreta (Farias et al., 2020). Due to the lack of studies on the effects of systemic fertilisation with phosphorus (P2O5) and potassium (K2O) in ICLS and cropping systems, (Farias et al., 2020) analysed ICLS under different fertilisation strategies in southern Brazil. Their study found that systemic fertilisation resulted in greater grass production without affecting soybean yield. Livestock production (in this case, sheep) led to a more efficient and productive system in terms of resource use. It is important to emphasise that the integration of crops and livestock does not harm the production system; on the contrary, these integrated systems, when well managed, are beneficial and important for global food production in the future (Farias et al., 2020).

Chapter 3

Objectives of the study

The main objective of this study is to investigate the nutrient dynamics and biomass production of soybean in no-till integrated crop-livestock systems under systemic fertilisation in southern in Brazil. In an attempt to achieve this objective from an experimental point of view, a study was carried out in the state of Rio Grande do Sul in southern Brazil during the soybean development cycle in ICLS and cropping system under systemic and conventional fertilisation. This study poses 2 hypotheses. The first is that soybean (*Glycine* max. (L.) Merr.) in no-till integrated crop-livestock systems under systemic fertilisation develops better agronomic characteristics throughout his development cycle. The second is that in no-till integrated crop-livestock system under systemic fertilisation, the dynamics of nitrogen, phosphorus and potassium (NPK) between the soil and the soybean plant (*Glycine* max. (L.) Merr.) are improved.

Chapter 4

Material and methods

I Field experiment

The experiment was conducted since 2017 at the Experimental Agronomic Station of the Federal University of Rio Grande do Sul (UFRGS), in Eldorado do Sul in southern Brazil. The regional climate according to the Köppen classification is humid subtropical (Cfa) (Alvares et al., 2013). The mean annual temperature and rainfall are 19,4°C and 1440 mm respectively. The soil in an Acrisol (Selcer, 2015) with low pH (5,3) and high Al saturation. The soil texture is sandy loam.

Before this experimentation, a preceding was implemented between 2003 and 2017 in an integrated crop-livestock system (ICLS), focusing on no-tillage practices. It examined various methods of stocking sheep grazing intensities on Italian ryegrass (*Lolium multiflorum* Lam.) in rotation with summer crops such as soybean and maize over a 14-year span. The management of pasture by moderate grazing intensities, called "Rotatinuous", is used in ICLS (Carvalho, 2013). Notably, the soil in the area was not treated for acidity; instead, it received fertilisation with nitrogen, phosphorus, and potassium, leading to a significant increase in acidification over time (Alves et al., 2019).

Since 2017, a randomized block design is implemented with a factorial 2 x 2 and four replicates (*Figure 4*). The factors studied were 2 no-till production system. The first one represented the ICLS and involved soybean cultivation in the crop phase and sheep grazing Italian ryegrass as a cover crop in the pasture phase (Grazed system). The second one, is a system with soybean cultivation in the crop phase and non-grazed Italian ryegrass as a cover crop in the pasture phase (Non-grazed system). Moreover, 2 different fertilisation periods for phosphorus (P2O5) with a dose of 35 kg/ha and potassium (K2O) with a dose of 50 kg/ha were considered. For the first modality, a conventional crop fertilisation were applicated, where fertilisers were applied during soybean sowing. For the second modality, a systemic fertilisation were used, where fertilisers were applied during pasture establishment.

A Nitrogen fertilisation (150 kg/ha) in the form of urea was applied during Italian ryegrass establishment across all treatments. The experimental area covered 4,4 ha and was divided in 16 experimental units (paddocks), each ranging between 0,23 and 0,32 ha, sufficiently large to prevent nutrient transfer between units. Pest control in the soybean crop was conducted on a weekly basis, and the use of herbicides, insecticides, and fungicides was carried out in accordance with the technical recommendations. The harvesting period typically occurs in April;

however, in the 2024 season, the occurrence of extreme rainfall at the conclusion of the cycle resulted in the inability to harvest, ultimately leading to a complete loss of production.

II Experimental design

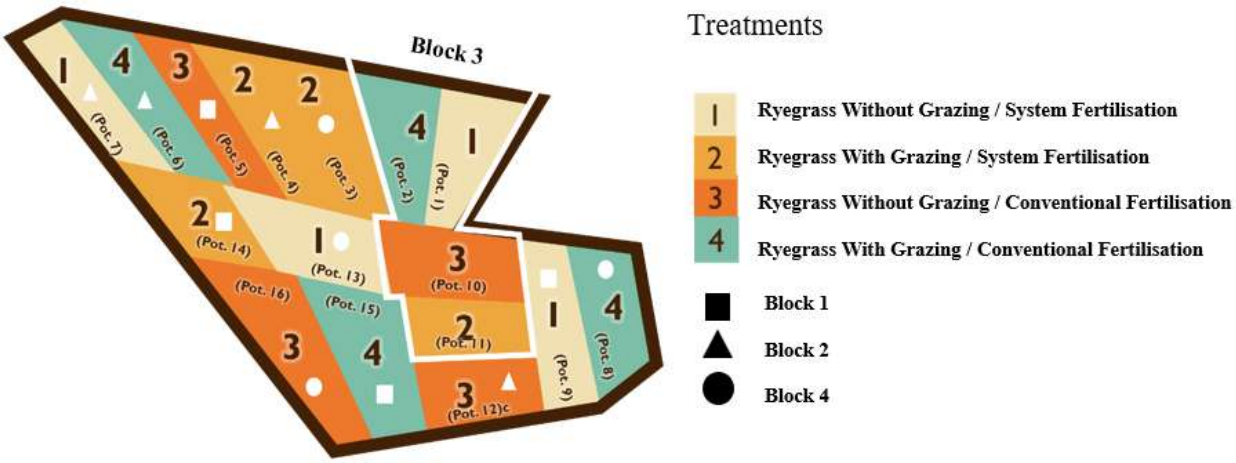


Figure 4: Experimental design of the experiment.

III Observations and variables measured

III.1 LAI

During 3 times in march, the LAI-2000 Plant Canopy Analyzer (LI-COR, Inc, Lincoln, Nebraska, U.S.A) was used to calculate LAI from light measurements taken above and below the canopy with a "fish-eye" optical sensor which simultaneously measures light interception at five zenith angles (LI-COR, Inc., 2024). 6 locations per plot were measured, each with a reference point above the canopy and 5 measurement points aligned under the plant canopy. The first date of collection was on 12 March at the R5.5 stage, the second on 22 March at the R6 stage and the 3rd five days later in the same stage. During this study, the leaf area index calculated, being a unitless parameter, will be used.

Figure 5: Remote control LAI 2000 plant canopy analyzer (LI-COR, Inc, Lincoln, Nebraska, U.S.A)

(a) Complete unit package.

(b) Device in field use.

Figure 6: Illustration of the LAI-2000 system in different contexts.

Light Transmission

The LAI-2000 measures the transmission of light through the plant canopy at different angles. The transmission (T) of light is calculated as follows:

$$T = \frac{I_{\text{below}}}{I_{\text{above}}}$$

where:

- I_{below} is the light intensity measured below the canopy.
- I_{above} is the light intensity measured above the canopy.

Leaf Area Index (LAI)

The LAI is calculated using the Beer-Lambert law for light extinction by the canopy:

$$I(z) = I_0 \exp(-K \cdot \text{LAI})$$

The LAI can be derived from this equation:

$$\text{LAI} = -\frac{\ln\left(\frac{I_{(z)}}{I_0}\right)}{K}$$

where:

- $I(z)$ is the light intensity at depth z in the canopy.
- I_0 is the light intensity at the top of the canopy.
- K is the extinction coefficient.
- LAI is the Leaf Area Index.

Extinction Coefficient (K)

The extinction coefficient (K) quantifies the attenuation of light as it passes through a plant canopy. It is influenced by the angle of light incidence, the arrangement and orientation of leaves, and the density and structure of the canopy. The extinction coefficient K is often approximated by $K = 0.5$ for horizontal light incidence and homogeneous canopies. The approximation of K as 0.5 can be theoretically justified by considering the geometry of leaf arrangement and the probability of light interception. In a randomly oriented and uniformly distributed leaf canopy, the average path length of light through the leaves results in an extinction coefficient that is approximately equal to this value (Gower & Norman, 1991).

III.2 Photosynthesis

During March, a LI-6400 XT (LI-COR, Inc, Lincoln, Nebraska, U.S.A) was used to measure various parameters related to the photosynthesis mechanism. The LI-6400 is an open system, which means that photosynthesis and transpiration measurements are based on the difference in CO2 and H20 concentration in an air stream passing through the leaf cuvette. The output data correspond to photosynthesis rate in μ mol of CO2 per unit area of intercepting leaf per unit time (μmol CO2/m^2/s).

The LI-6400 represents a significant advancement over conventional open-system systems, primarily due to the integration of gas analyzers within the sensor head. This configuration eliminates time lags associated with plumbing, thereby enabling precise and rapid control in response to foliar fluctuations. For instance, the instantaneous closure of stomata is promptly detected by the system as a decrease in water vapor, facilitating immediate compensatory measures. Similarly, an abrupt alteration in light intensity triggers a immediate change in the rate of photosynthesis, which is discerned as a shift in CO2 concentration. Notably, the velocity of detection is independent of the system's flow rate, a characteristic that contrasts with traditional systems, given the presence of the sample IRGA (infrared gas analyzer) within the cuvette.

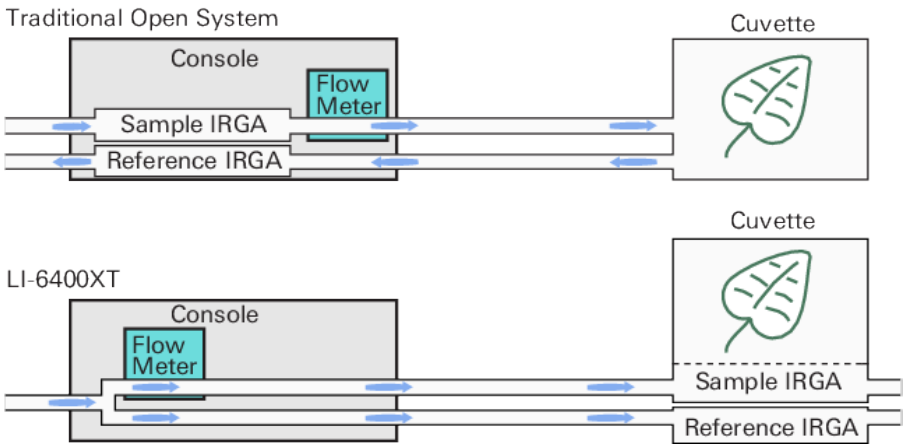


Figure 7: Particularity of LI-6400 XT system.

Furthermore, the placement of the IRGAs within the sensor head offers an additional advantage. In traditional systems, there is a potential for concentration changes, attributable to water sorption and CO2 diffusion, as the air traverses from the reference IRGA to the chamber, and subsequently, from the chamber to the sample IRGA. However, this issue is circumvented in the LI-6400, as the IRGA measurements are conducted following the passage of air through the tubing.

The LI-6400 XT was employed to gather data over a 4-day period during the growth phase of the soybeans present in the experimental plots. For each plot, a total of 2 leaves were subjected to analysis using the LI-6400 XT. To ensure the selection of a healthy leaf with a representative photosynthetic rate, a criterion based on the 3rd youngest leaf was applied. The data collection sequences were executed in a block-by-block manner, with one sequence conducted in the morning and another in the afternoon on each day. The collection protocol followed the user manual provided by the company. The 4 samples were spread over 3 stages. The first on 6 March at the R5.4 stage, the second on 23 March at the R6 stage, the 3rd on 28 March at the same stage and the 4th on 3 April at the R7 stage. During this study, the net rate of photosynthesis expressed in µmol CO2 m^{-2} s^{-1} calculated by the device will be used.

(a) Unit dismantled in storage box. **(b)** Device ready for use.

Figure 8: LI-6400XT (LI-COR, Inc, Lincoln, Nebraska, U.S.A) before and during use.

III.3 Biomass

The biomass and nutrient study was designed during three distinct soybean stages: R1, R5.5 and R8, illustrate in *Figure 9*. To achieve this, a systematic data collection protocol was implemented, ensuring data consistency and accuracy at the different stages of plant development. The following sections describe the methods used to collect the data required for each growth stage.

Six soybean plants were randomly sampled per paddock at each stage. Care was taken to handle the plants delicately to avoid damage. At stage R1, the roots were also removed. For the final R8 stage, i.e. the mature stage of the plant, the 6 samples per paddock were collected, along with the leaves on the ground at the base of each sample. A 19 x 19 cm quadrat was used to collect several leaves corresponding to those that had fallen from the plant. With an average of 12.5 plants per metre and a row spacing of 0.45 m, the number of plants per hectare was approximately of 278,000. The 0.036 m^2 quadrat therefore represents the space occupied by one plant on the soil surface. The sum of the winds was also considered to be homogeneous.

Subsequently, the sampled plants underwent drying in an oven at a temperature of 55°C for at least 72 hours until their weight stabilized. After drying, the plants were carefully dissected to separate for the two last stages (R5.5 and R8) the pods and the vegetative biomass and for the first stage (R1) the aerial part and the root. The pods were then counted to assess pod development and at stage R1, the nodules were separated from the roots and counted also.

The biomass of each plant organ was then determined using a LG163 precision balance (BEL Engineering srl, Monza, Italy).

(a) R1 **(b)** R5.5 **(c)** R8

Figure 9: Photo of soybean plants at each stage of collection.

III.4 Nutrients

For laboratory analysis of macronutrients content (NPK), samples of R1 stage were prepared by pairing them in sets of two (samples 1-2, samples 3-4, samples 5-6). This pairing ensured a sufficient quantity of crushed material for titration. Concerning stages R5.5 and R8, for pods and vegetative tissue, 3 samples were selected for grinding (samples 1, samples 3, samples 5). This ensured that multiple locations within each paddock were represented for analysis.

The samples were ground to a fine powder with a TE-650 from (Tecnal, Piracicaba - SP, Brazil) to facilitate nutrient extraction and analysis (*Figure 10a*). Laboratory analysis was conducted to determine the levels of nitrogen, phosphorus, and potassium present in the aboveground parts of the plants. A method of sample digestion by adding H_2SO_4 at high temperature were used. The mixture is homogenised with compressed air (using a laboratory pump) and after decantation (6-12 hours), aliquots of the extract are taken for the various determinations. Standardized protocol from Tedesco J. M. et al (1995) was followed to ensure accuracy and reliability in nutrient analysis. Scans of the sections on plant residue analysis have been added to the appendices (*Appendix I*). The laboratory analyses were carried out by the fertility laboratory of the Secretariat of Agronomy of the state of Rio Grande do Sul.

(a) Grinder used. **(b)** Grinded plants for laboratory analysis.

Figure 10: Overview of the grinder and crushed plant samples.

III.5 Soil

In parallel with the plant collections, soil samples were organized. Three samples per paddock were collected, which were then mixed, loosened, and homogenized to analyze the mineral and organic nitrogen present. To ensure synchronous analyses, four collections of soil of 10 cm deep were selected for analysis. Among these, three were conducted at key reproductive stages (R1, R5.5, and R8).

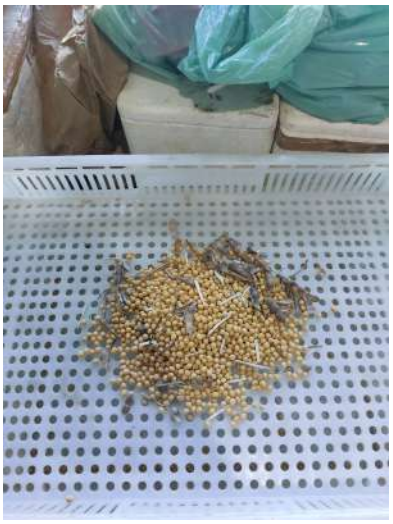
To extract total and mineral nitrogen from the collected soil, preparation was required. Firstly, the soil was moisturised and dried at 100 degrees in an oven for 48 hours. Part of this soil is frozen. 5 grams of fresh soil is added to 50 ml of a 1 molar solution of KCL. The solution is agitated for 30 min. The mixture is then filtered and the extract sent for distillation. Standardized protocol from Tedesco J. M. et al (1995) was followed to ensure accuracy and reliability in nutrient analysis. Scans of the sections on soil nitrogen analysis have been added to the appendices (*Appendix II*). The analyses were carried out by the fertility laboratory of the Secretariat of Agronomy of the state of Rio Grande do Sul.

IV Yield study

At the very end of the experiment, before harvesting, a productivity study was carried out. For this purpose, a final collection was organized to measure the yield parameters of the experimental plot. For each paddock, 6 samples of plants were harvested, totaling 96 plants. During each sampling, plants present on 2 meters of row were collected and counted. Knowing the number of plants on 2 meters allowed for an approximation of the number of plants per hectare.

After weighing the total biomass of each sample and counting the number of pods, the plants were processed using a sorter to extract the grains from the pods. A sieve was then used for more precise sorting. The weight, number of grains, and moisture content of each sample were measured. A SL95 Moisture meter from Steinlite was used to measure grain moisture content. This measurements allowed for the calculation of several parameters, such as the thousand-grain weight, the number of grains per pod, the harvest index (grain biomass/total plant biomass), and productivity.

(a) Sorter used.



(b) Grains before sieving.

(c) Grains after sieving.

Figure 11: Elements of the soybean bean sorting process.

Figure 12: Steinlite SL95 Moisture meter.

V Variable listing

According to following *Table 4.1*, the variables collected are described and linked to the harvest stage. Due to different collection dates, Leaf area index (LAI) and net photosynthetic rate collections have not been added to the table, nor has the 4th collection stage for soil variables.

Table 4.1: List of variables with their descriptions and corresponding stages for data collection.

Variable	**Description**	**Stage R1**	**Stage R5.5**	**Stage R8**
Nodule number	Number of nodules per plant	✓	□	□
Pod number	Number of pods per plant	□	✓	✓
Nodule biomass (g)	Nodule biomass per plant in grams	✓	□	□
Pod biomass (g)	Pod biomass per plant in grams	□	✓	✓
Root biomass (g)	Root biomass per plant in grams	✓	□	□
Vegetative biomass (g)	Vegetative biomass in grams	✓	✓	✓
N% leaves + stem	N content of vegetative parts in %	✓	✓	✓
P% leaves + stem	P content of vegetative parts in %	✓	✓	✓
K% leaves + stem	K content of vegetative parts in %	✓	✓	✓
N leaves + stem (g)	N amount in vegetative parts in grams	✓	✓	✓
P leaves + stem (g)	P amount in vegetative parts in grams	✓	✓	✓
K leaves + stem (g)	K amount in vegetative parts in grams	✓	✓	✓
N% pod	N content of pods in %	□	✓	✓
P% pod	P content of pods in %	□	✓	✓
K% pod	K content of pods in %	□	✓	✓
N pod (g)	N amount in pods in grams	□	✓	✓
P pod (g)	P amount in pods in grams	□	✓	✓
K pod (g)	K amount in pods in grams	□	✓	✓
N plant (g)	N amount in the plant in grams	✓	✓	✓
P plant (g)	P amount in the plant in grams	✓	✓	✓
K plant (g)	K amount in the plant in grams	✓	✓	✓
N Total soil (g/kg)	N total amount per kg of soil in grams	✓	✓	✓
NO3 soil (g/kg)	NO3 amount per kg of soil in grams	✓	✓	✓
NH4 soil (g/kg)	NH4 amount per kg of soil in grams	✓	✓	✓

VI Events influencing data collection

Although the experimental conditions were carefully chosen to be as close as possible to field conditions, space, time and budget constraints influenced the measurements of the study.

First and foremost, access to equipment to measure LAI and photosynthesis rates limited the collection period. The equipment used was supplied by the agronomy department of the partner university and required considerable time to learn and understand how to use. As a result, it could only be used from the R5.4 stage of the soybean. The weather also had an impact on the use of the LI-6400 XT (LI-COR, Inc, Lincoln, Nebraska, USA). High temperatures prevented the LI-6400 XT from working properly by blocking the calibration of the IRGA.

Then, it is important to note that the dates for collecting photosynthetic parameters depend on the soybean cycle. Leaf area and net photosynthetic rate decrease throughout the cycle. As soybean plants lose leaves during late growth, canopy density decreases, which changes the way light is diffused and transmitted through the canopy. The LAI 2000 Plant Canopy Analyzer (LI-COR, Inc, Lincoln, Nebraska, U.S.A.) is an instrument that requires a certain leaf density to be measured in order to obtain accurate light transmission results. At too advanced a stage, the leaf density of soybean plants is too low to make relevant and accurate leaf area measurements. Under these conditions, the "fish-eye" optical sensor does not discriminate significantly between the incident rays above the canopy and those below the canopy (Jonckheere et al., 2004). The LI-6400 XT requires the selection of healthy, photosynthetically active leaves. As leaves begin to fall, it becomes difficult to select representative, healthy leaves for measurement. The remaining leaves may be damaged, senescent or dying, affecting the measured photosynthetic rates. In addition, the availability of healthy leaves for measurement decreases, making repeated and representative measurements more difficult (Kar et al., 2021).

Finally, the laboratory analysis to obtain the soil mineral nitrogen and the macronutrients present in the different plant organs took longer than expected. Indeed, extreme events occurred at the end of April throughout the Rio Grande do Sul region, preventing any travel to Porto Alegre, where the analysis laboratories are located. Additionally, these conditions trapped the student at the Experimental Agronomic Station of the Federal University of Rio Grande do Sul (UFRGS), preventing him from conducting the analyses.

VII Statistical analysis

All statistical analyses were performed on Rstudio in R language (version 4.3.1) and the packages used grouped together in *Table 4.2*. All samples were assumed to be random, simple, and independent. All plant biomass and yield data have a n = 24. The plant nutrient data, as explained above, have an n = 12. All soil data, having already been averaged, have an n = 4 (mean per paddock).

A first analysis of variance is performed with comparisons between the two fixed factors. The first one is the pasture modality CP and SP (Grazed and Non-grazed paddocks) and the second one is the fertilisation type AI and AV (Systemic and Conventional). An lme model has been built for each stage to incorporate the fixed factors and the random paddock factor. ANOVA's test type III of the lme model were performed, as well as Shapiro-Wilk and Bartlett tests to check the normality and homoscedasticity of the residuals. For all the analyses of variance, the significance level was P <0.05, P <0.01 being highly significant and P <0.001 very highly significant. Where there was significance, Tukey's multiple comparison test of the estimated marginal means (emmeans function) pairwise were used to observe the effects of each modality group on the dependent variables. For each treatment, a comparison was made between the modalities of the other treatment. For example, under the CP treatment, a comparison was made between AI and AV. The same was done for the SP treatment and for the two fertilisation treatments. These tests revealed some significant differences between groups.

In addition, GGpairs of the variables of interest were generated. One for the global correlation and one by factor combination (Grazed-systemic, Non-grazed-systemic, Grazed-conventional, Non-grazed-conventional). As a further development, Pearson tests and linear regressions were performed on the significant correlations retained. To conclude, a discriminant analysis was carried out with the function "lda". This analysis makes it possible to simplify the data while retaining as much information as possible about the differences between classes. Histograms were produced to facilitate interpretation and represent the contribution of the variables for each discriminant.

Package	Version	Package	Version
readxl	1.4.3	ggplot2	3.5.1
patchwork	1.2.0	ggstatsplot	0.12.3
factorextra	1.0.17	dplyr	1.1.4
nlme	3.1-164	FactoMineR	2.11
factoextra	1.0.7	GGally	2.2.1
multicomp	1.4-25	ggrepel	0.9.1
gridExtra	2.3	gridGraphics	0.5-1
emmeans	1.9.3	car	3.1-2
corrplot	0.92	tidyverse	2.0.0
brms	2.21.0	ggfortify	0.4.17

Table 4.2: R packages used.

Chapter 5

Results

I Study by stage

The purpose of these section is to provide answers to the hypotheses by analysing the variables of biomass and nutrient measured in the key stage of this study. The results of statistical analyses carried out and boxplots of significant effects are exposed. Tables of averages and standard deviations are presented in the appendix (*Table B.1, Table B.2, Table B.3*).

I.1 Beginning flowering stage (R1)

Neither the pasture factor nor the fertilisation factor showed significant effects on most of the variables studied for the soybean flowering stage. However, the fertilisation has an impact in K content of the vegetative parts (leaves + stem) (*Table 5.1*). The multiple comparison test did not reveal any differences between the groups of means (by combination of factors) for this variable but *Figure 14* shows that the plants in systemic fertilisation grazed plots are less rich in potassium than those in the other treatments. In fact, K% of the vegetative parts is on average 0.333% lower than in the 3 other conditions. For the soil variables, the pasture treatment increased the quantities of both NH4 and NO3 in the first soil horizon ($p = 0.04$). The comparison test concluded that there was a significant difference between NH4 in the grazed plots and in the non-grazed plots under systemic fertilisation (*Figure 13*). The graphic also shows the significance of pasture factor on the NO3 variable. For systemic fertilisation, a difference of 0.305 g/kg between the two pasture modalities is observed.

Table 5.1: Results of ANOVA test of biomass and nutrient levels of the various soybean organs and soil nitrogen levels studied at the beginning of flowering (R1 stage) under grazing and fertilisation treatments.

R1	Variables	P-values		
		Pasture	Fertilisation	Interaction
Plant	Nodule number	0.859	0.661	0.901
	Nodule biomass (g)	0.519	0.666	0.647
	Root biomass (g)	0.231	0.207	0.435
	Vegetative biomass (g)	0.344	0.338	0.435
	N% leaves + stem	0.935	0.726	0.404
	P% leaves + stem	0.933	0.274	0.312
	K% leaves + stem	0.112	0.041*	0.258
	N leaves + stem (g)	0.406	0.391	0.127
	P leaves + stem (g)	0.561	0.382	0.396
	K leaves + stem (g)	0.933	0.969	0.725
Soil	N Total soil (g/kg)	0.366	0.866	0.194
	NO3 soil (g/kg)	0.040*	0.974	0.197
	NH4 soil (g/kg)	0.035*	0.966	0.239

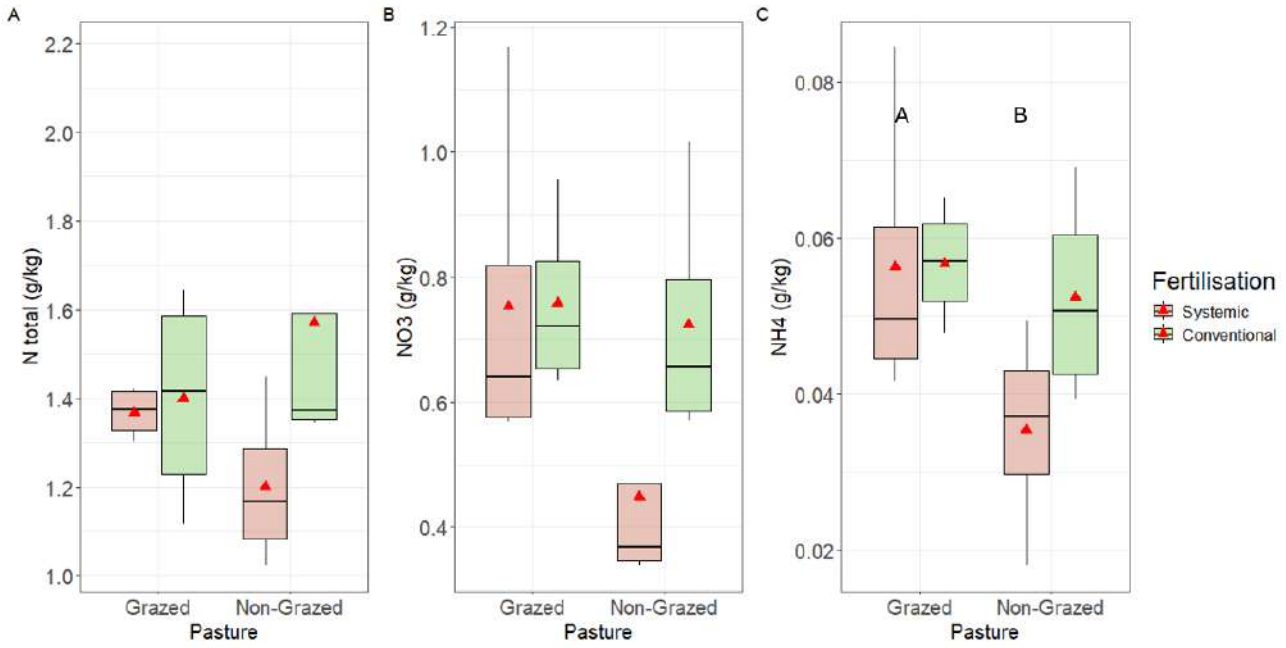


Figure 13: Boxplots of nitrogen in the soil at begin flowering stage by factor combination with the representation of the mean (△), the significant difference of multiple comparison test between grazing modalities is represented by the letters "A" and "B".

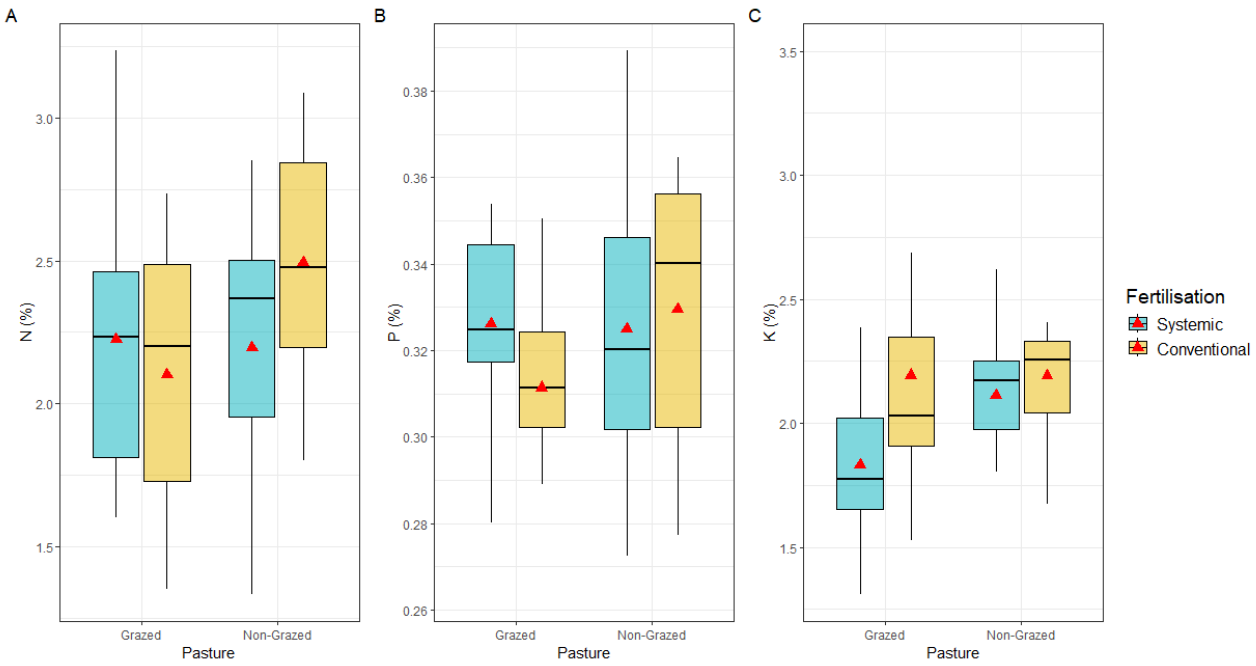


Figure 14: Boxplots of plant nutrient content at begin flowering stage by factor combination with the representation of the mean (△).

I.2 End of grain filling stage (R5.5)

Neither the pasture factor nor the fertilisation factor showed significant effects on most of the variables studied, as shown in *Table 5.2* for the soybean at end filling grain stage. However, the pod variables are sensitive to grazing (p= 0,019 and 0,026). The multiple comparison test reveals differences between the pasture modalities under systemic fertilisation for pod number and pod biomass (p= 0,038 and 0.04) represented in *Figure 15* with "A" for the highest mean and "B" for the lowest one. Furthermore, this test shows significant differences in pod number between the fertilisation modalities for the non-grazed paddocks (p= 0,039) represented in *Figure 15* with "a" for the highest mean and "b" for the lowest one. The ICLS have a significant higher mean of pod number and pod biomass under systemic fertilisation. However, systemic fertilisation leads to a lower number of pods in plots that are not grazed. Plant phosphorus amount are higher of 0,053 g in grazed plots under systemic fertilisation, but the multiple comparison test did not reveal any significant difference between the groups.

The significance of the pasture factor and the interaction between the 2 factors in the N Total soil variable but not in the other soil variables led us to carry out an ANOVA test on the N organic variable calculated as the difference between N Total and NH4 + NO3. This test confirms the interaction between the factors and the significant effect of the grazing factor. The p-value of 0.03 of the multiple comparison test and *Figure 16* illustrate the significant difference between the grazed and non-grazed conditions for systemic fertilisation. Under systemic fertilisation, N orga variable in non-grazed paddocks is almost twice as high than in the grazed paddocks (0.908 g/kg and 0,468 g/kg).

Table 5.2: Results of ANOVA test of biomass and nutrient levels of the various soybean organs and soil nitrogen levels studied at the end of grain filling (R5.5 stage) under grazing and fertilisation treatments.

R5.5	Variables	P-values		
		Pasture	Fertilisation	Interaction
Plant	Pod number	0.019 *	0.610	0.046 *
	Pod biomass (g)	0.026 *	0.696	0.269
	Vegetative biomass (g)	0.057.	0.638	0.344
	N% pod	0.220	0.457	0.832
	P% pod	0.299	0.973	0.773
	K% pod	0.286	0.196	0.421
	N pod (g)	0.195	0.793	0.520
	P pod (g)	0.177	0.984	0.619
	K pod (g)	0.447	0.696	0.647
	N% leaves + stem	0.487	0.629	0.673
	P% leaves + stem	0.399	0.689	0.777
	K% leaves + stem	0.278	0.209	0.920
	N leaves + stem (g)	0.431	0.932	0.628
	P leaves + stem (g)	0.107	0.840	0.346
	K leaves + stem (g)	0.906	0.375	0.588
	N plant (g)	0.235	0.836	0.527
	P plant (g)	0.044 *	0.876	0.324
	K plant (g)	0.722	0.450	0.588
Soil	N Total soil (g/kg)	0.033 *	0.101	0.016 *
	NO3 soil (g/kg)	0.133	0.295	0.197
	NH4 soil (g/kg)	0.179	0.136	0.164

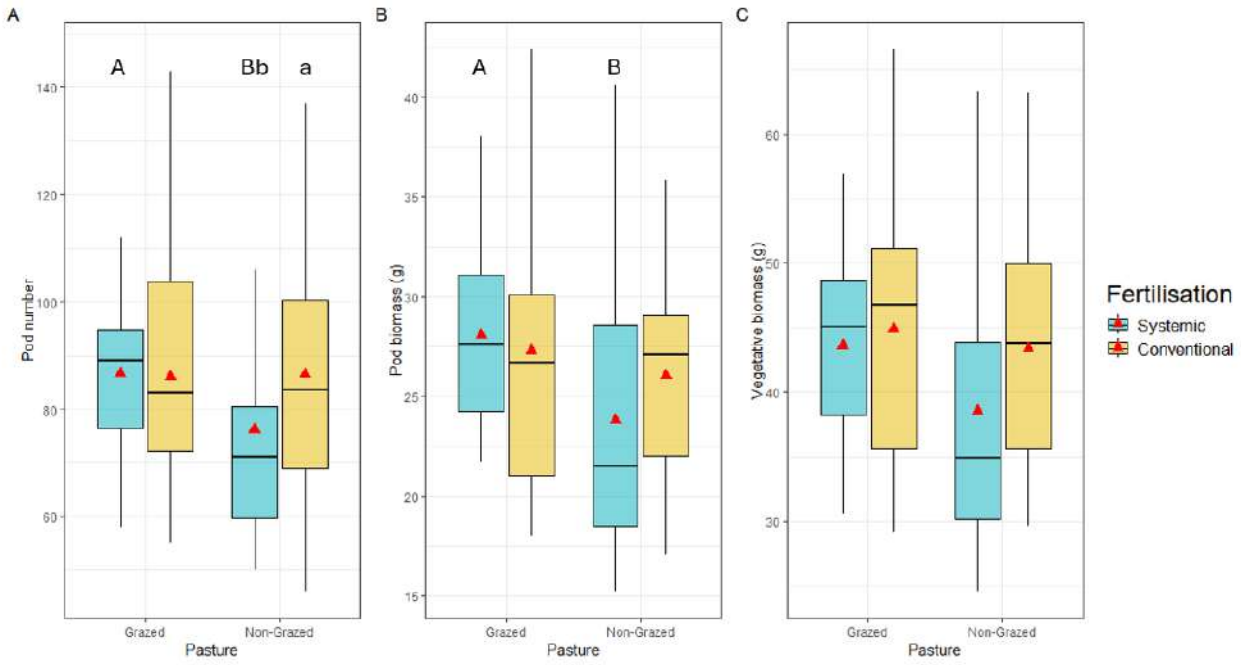


Figure 15: Boxplots of biomass characteristics at end grain filling stage by factor combination with the representation of the mean (△), the significant difference of multiple comparison test between grazing modalities is represented by the letters "A" and "B" and between the fertilisation modalities by the letters "a" and "b".

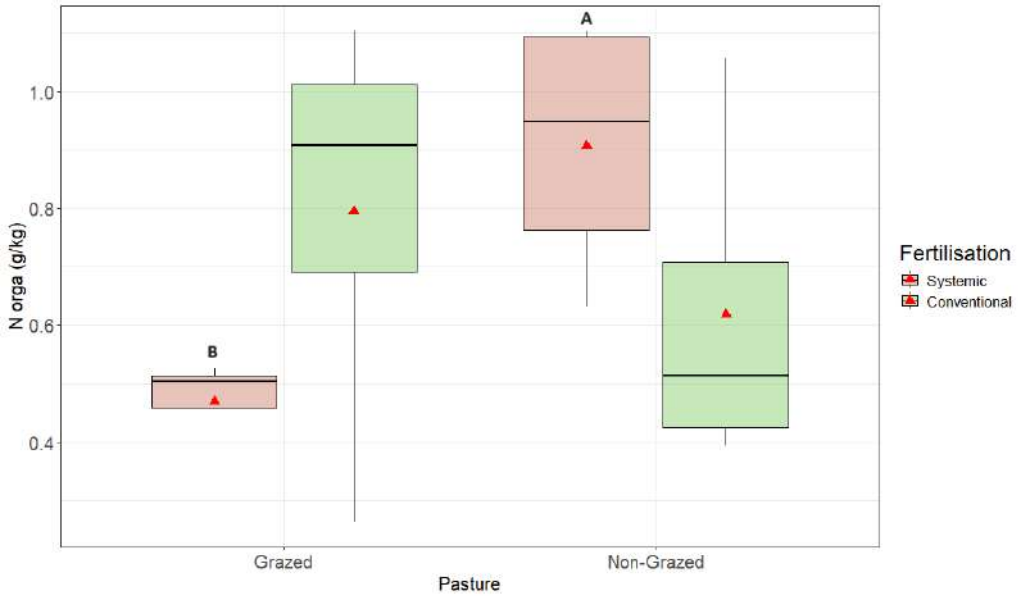


Figure 16: Boxplots of organic N in the soil at R5.5 stage by factor combination with the representation of the mean (△), the significant difference of multiple comparison test between grazing modalities is represented by the letters "A" and "B".

I.3 Maturity stage (R8)

Neither the pasture factor nor the fertilisation factor showed significant effects on most of the variables studied, as shown in *Table 5.3* for the soybean at maturity stage. However, the pod variables are sensitive to grazing (p= 0,004 and 0,022). The multiple comparison test reveal differences between the pasture modalities under systemic fertilisation for pod number and pod biomass (p= 0,040 and 0.032) represented in *Figure 17* with A for the highest mean and B for the lowest one. The same multiple comparison test results were noted for total biomass and the nitrogen and potassium content of the vegetative parts. The grazing using systemic fertilisation has a significant impact on these variables. Furthermore, this constrast test shows significant differences between the fertilisation modalities for the grazed paddocks (p= 0,035) represented in *Figure 18* with △ for the highest mean and ▽ for the lowest one. The ICLS have a significant higher mean of pod number, pod biomass, total biomass and N content in vegetative parts under systemic fertilisation. Moreover, systemic fertilisation leads to higher pod biomass in grazed paddocks. Under systemic fertilisation, plant potassium content are significantly lower in the grazed plots. The effect of pasture on NH4 in the first 10 cm of soil is close to significance in ANOVA.

Table 5.3: Results of ANOVA test of biomass and nutrient levels of the various soybean organs and soil nitrogen levels studied at maturity (R8 stage) under grazing and fertilisation treatments.

R8	Variables	P-values		
		Pasture	Fertilisation	Interaction
Plant	Pod number	0.004 **	0.036 *	0.102
	Pod biomass (g)	0.022 *	0.018 *	0.037 *
	Vegetative biomass (g)	0.139	0.091	0.262
	Total biomass (g)	0,031*	0.127	0.241
	N% pod	0.349	0.110	0.795
	P% pod	0.484	0.461	0.621
	K% pod	0.923	0.657	0.908
	N pod (g)	0.332	0.413	0.935
	P pod (g)	0.311	0.533	0.985
	K pod (g)	0.368	0.690	0.997
	N% leaves + stem	0.006 **	0.364	0.108
	P% leaves + stem	0.586	0.116	0.716
	K% leaves + stem	0.016 *	0.012 *	0.016 *
	N leaves + stem (g)	0.408	0.702	0.476
	P leaves + stem (g)	0.267	0.388	0.884
	K leaves + stem (g)	0.203	0.146	0.218
	N plant (g)	0.251	0.422	0.881
	P plant (g)	0.238	0.976	0.958
	K plant (g)	0.895	0.616	0.540
Soil	N Total soil (g/kg)	0.743	0.379	0.520
	NO3 soil (g/kg)	0.070	0.599	0.162
	NH4 soil (g/kg)	0.057.	0.303	0.143

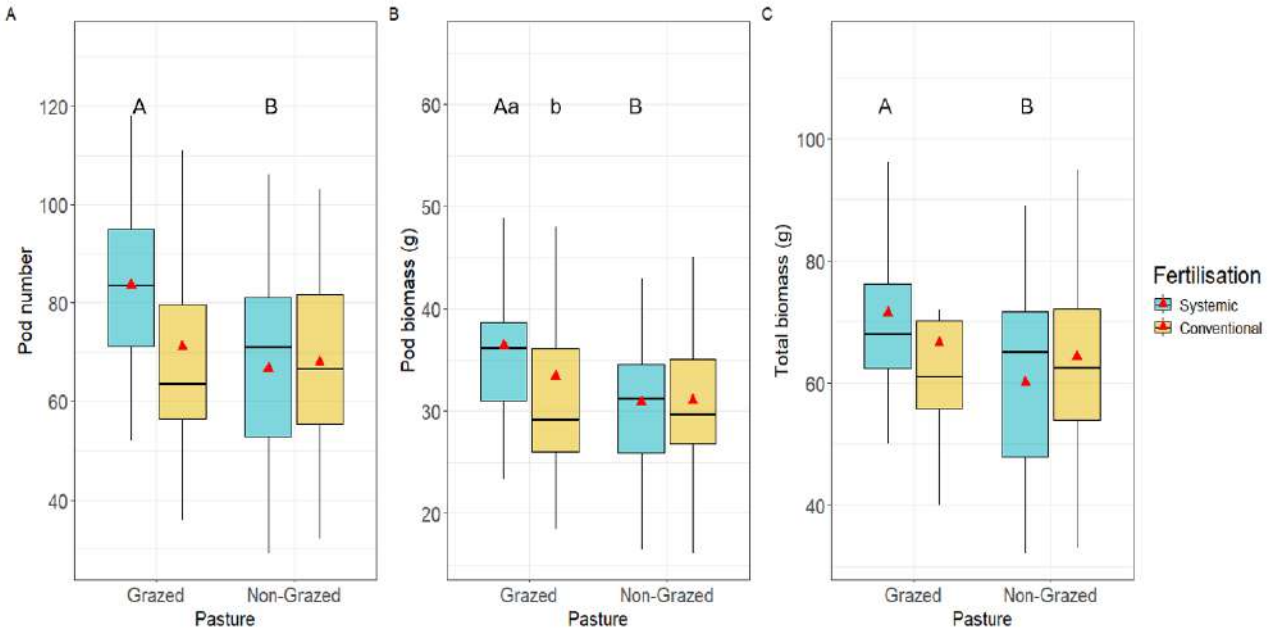


Figure 17: Boxplots of plant biomass characteristics at maturity stage by factor combination with the representation of the mean (△), the significant difference of multiple comparison test between grazing modalities is represented by the letters "A" and "B" and between the fertilisation modalities by the letters "a" and "b".

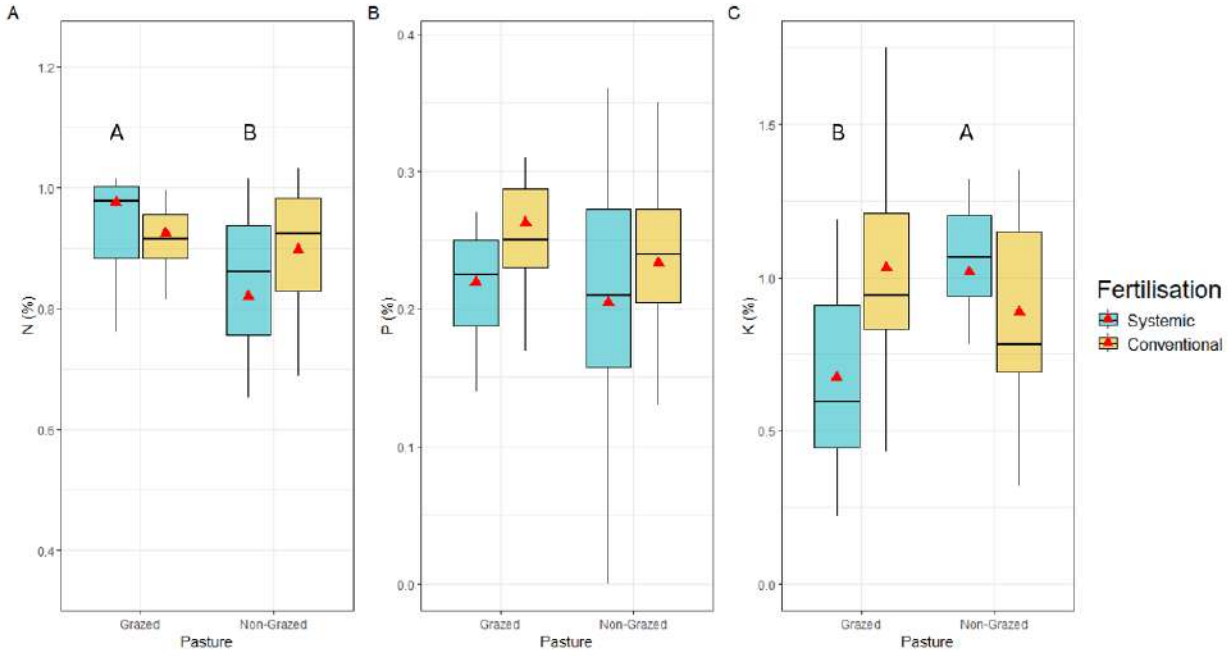


Figure 18: Boxplots of leaves and stem nutrients (%) at maturity stage by factor combination with the representation of the mean (△), the significant difference of multiple comparison test between grazing modalities is represented by the letters "A" and "B".

II Yield study

No effects of the grazing treatment or the fertilisation method on the thousand weight grains which ranged between 167 and 172 grams has been noticed (*Figure 19*). Similarly, the treatments don't influence the total soybean yield which ranged from 5490 to 5979 kg/ha. Table of averages and standard deviations are exposed in the appendix (*Table B.4*).

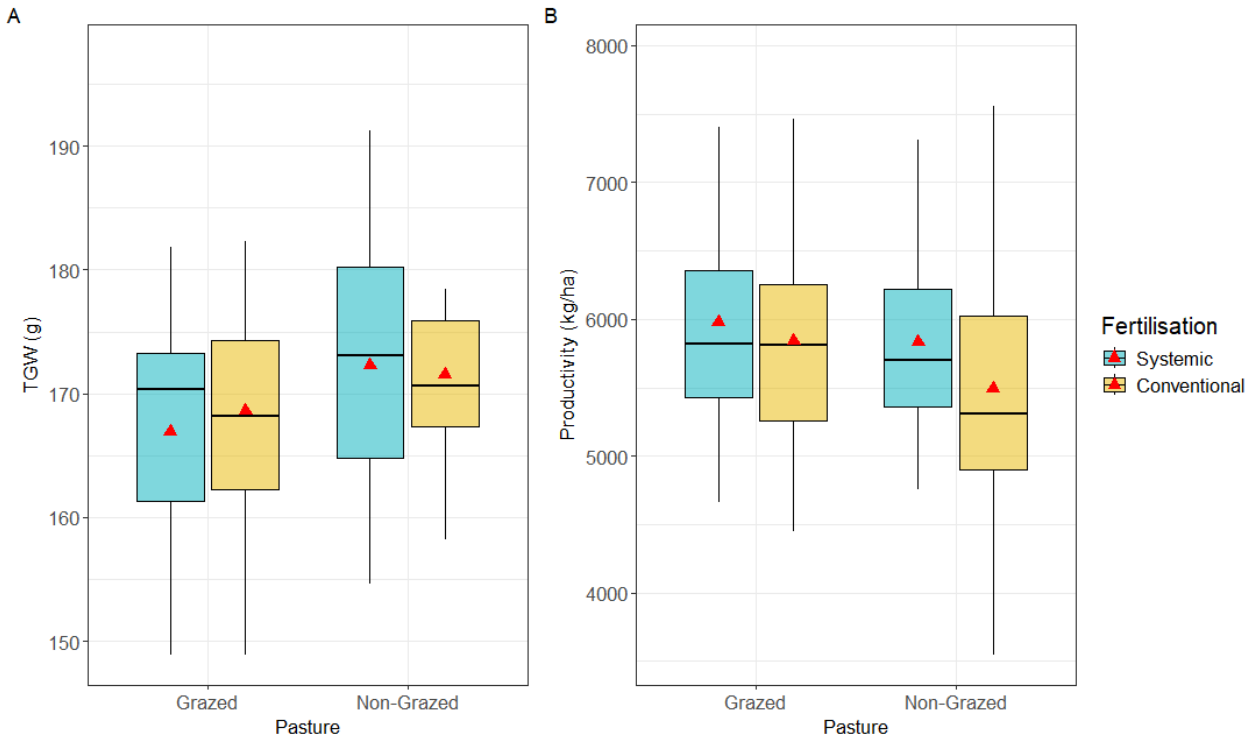


Figure 19: Boxplots of plant biomass characteristics at maturity stage by factor combination with the representation of the mean (△).

III Global evolutions

This section presents the results of studies of changes in the various parameters measured over time and the results of statistical analyses of LAI and net photosynthetic rate of stages measured. Tables of averages and standard deviations are exposed in appendix (*Table B.5, Table B.6*).

III.1 LAI

Below are the results of the ANOVA and multiple comparison tests carried out on the leaf area index, making it possible to observe the evolution of senescence at the end of the soybean cycle. There is an increase in the significance of the pasture factor over time on LAI (*Table 5.4*). The grazing factor has a significant effect on the LAI variability. It is very highly significant on 22 March and 27 March with a power of 10 difference in the p-value between the two collects. At the first stage, the average LAI for the grazed conditions, regardless of the type of fertilisation, is lower than for the non-grazed modality but the p-value of ANOVA is not significant. Thereafter, however, a clear superiority emerged, especially for systemic fertilisation (*Figure 20*). Whatever the method used, the leaf area index decreases over time.

Table 5.4: Results of ANOVA for LAI at each stage under grazing and fertilisation treatments.

Stage	P-values		
	Pasture	Fertilisation	Interaction
12-march (R5,5)	0.234	0.676	0.466
22-march (R6)	0.0004***	0.137	0.226
27-march (R6+5D)	$3.93 * 10^{-10}$***	0.06	0.226

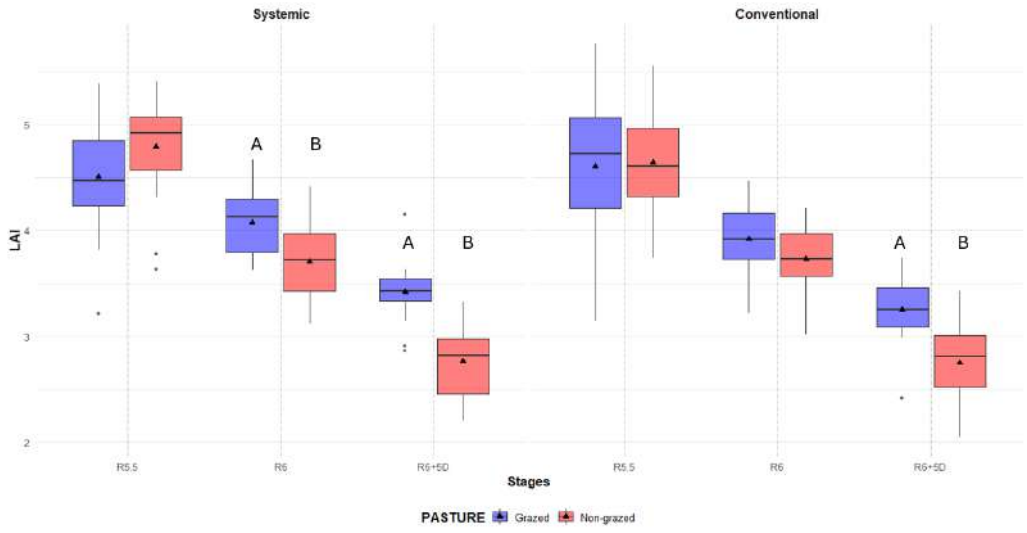


Figure 20: Boxplots of LAI at each stage of collect by factor combination with the representation of the mean (△), the significant difference of multiple comparison test between grazing modalities is represented by the letters "A" and "B".

III.2 Photosynthesis

Below are the results of the ANOVA and multiple comparison tests carried out on the photosynthesis rate of the plants, making it possible to observe changes in the physiological health of the plants. The results of the analysis of variance tests on the photosynthetic rate of soybean plants show an increase in the significance of the Pasture factor over time. The grazing factor has a significant effect on the photosynthetic rate in the last 3 collections. It was highly significant on 23 March and very highly significant on 28 March and 3 April. The significance of fertilisation at the R6 stage can be seen in *Table 5.5*. The mean photosynthetic rates at the R6 stage differ markedly between the two fertilisation modalities for the grazed conditions. After this stage, the means of grazed conditions are significantly higher than those of the non-grazed modality (*Figure 21*). Just like the LAI variable, the photosynthetic rate decreases over time.

Table 5.5: Results of ANOVA for photosynthetic rate (µmol/m^2/s).

Stage	P-values		
	Pasture	**Fertilisation**	**Interaction**
06-march (R5.4)	0.3751	0.3420	0.5591
23-march (R6)	0.004**	0.01*	0.156
28-march (R6+5D)	$1.91 * 10^{-9}$***	0.104	0.761
03-april (R7)	$1.19 * 10^{-10}$***	0.895	0.938

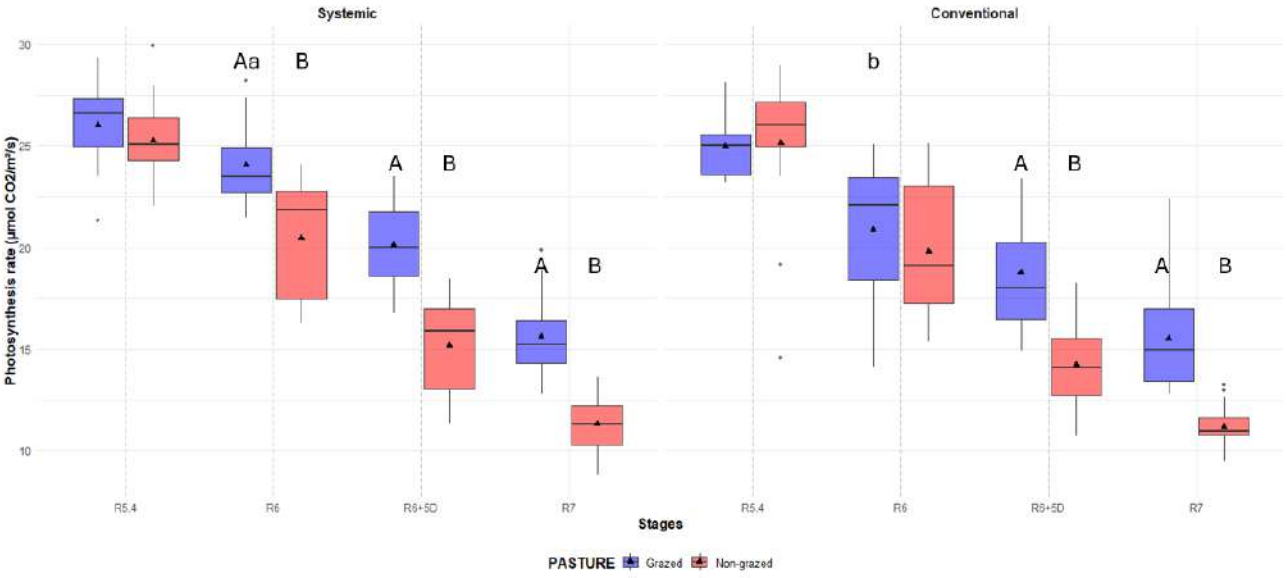


Figure 21: Boxplots of photosynthesis rate (µmol CO2/m^2/s) by factor combination with the representation of the mean (△), the significant difference of multiple comparison test between grazing modalities is represented by the letters "A" and "B" and between fertilisation modalities by the letters "a" and "b".

III.3 Pod Nitrogen

Nitrogen levels in pods increase between the last two stages studied for plots with systemic fertilisation (*Figure 22*). The opposite trend can be observed for paddocks under conventional fertilisation. The grazed-systemic fertilisation results in a nitrogen content close to 1.75 grams per plant on average at maturity, whereas under the other modalities, pod nitrogen does not exceed 1.5 grams at this final stage.

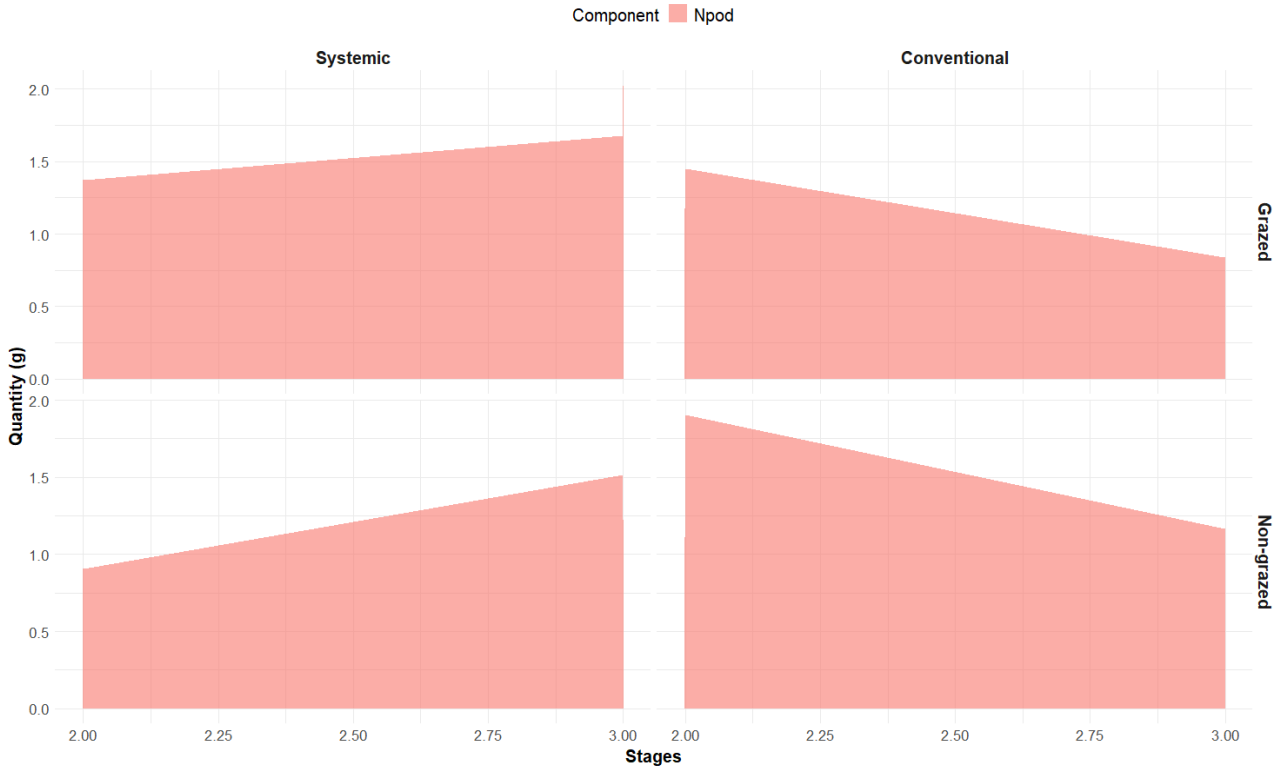


Figure 22: Evolution in nitrogen amount means (g) in soybean plant in the four soybean systems and the three collect stages by grazed and fertilisation modalities. 2.00 = end of grain filling (R5.5) and 3.00 stage = maturity (R8).

III.4 Plant Nutrients

Total nitrogen per plant in the grazed-systemic system is higher at each stage, whereas in conventional fertilisation it is more variable. *Figure 23* illustrates the evolution of the total nitrogen contained in soybean plants. We can see an increase in the last two stages for systemic fertilisation and a decrease for conventional fertilisation. Moreover, the nitrogen quantity values are higher for the grazed modality up to 2 grams for the grazed-systemic combination. However, no significant effect of factors was observed.

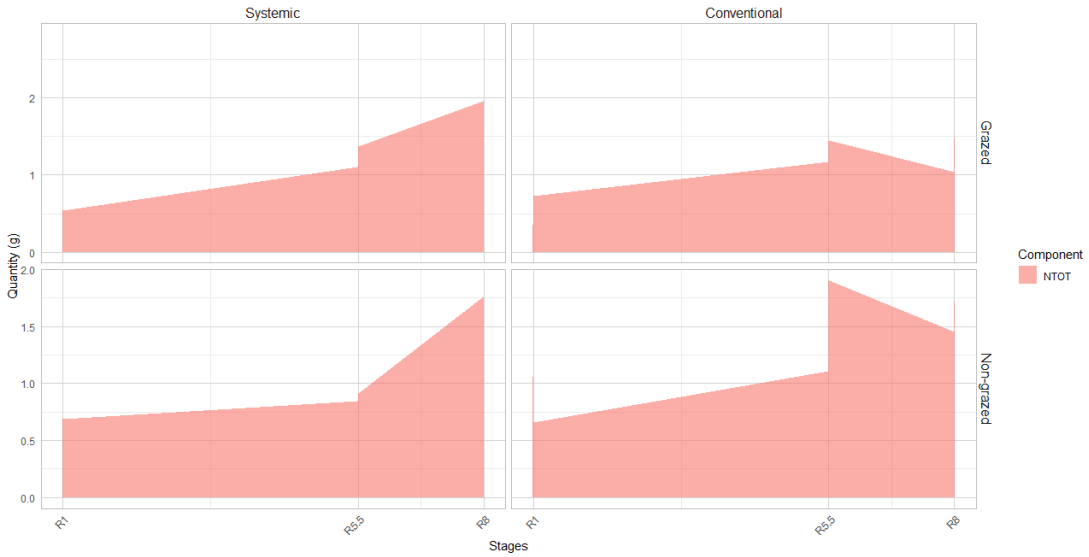


Figure 23: Evolution in nitrogen amount (g) in soybean plant in the four soybean systems and the three collect stages by factor combination.

III.5 Soil

Figure 24 and 25 clearly show a higher average for NH4 and NO3 for the grazed-systemic fertilisation combination in all the cycles studied. The opposite is clearly evident for organic nitrogen, as shown in *Figure 26*.

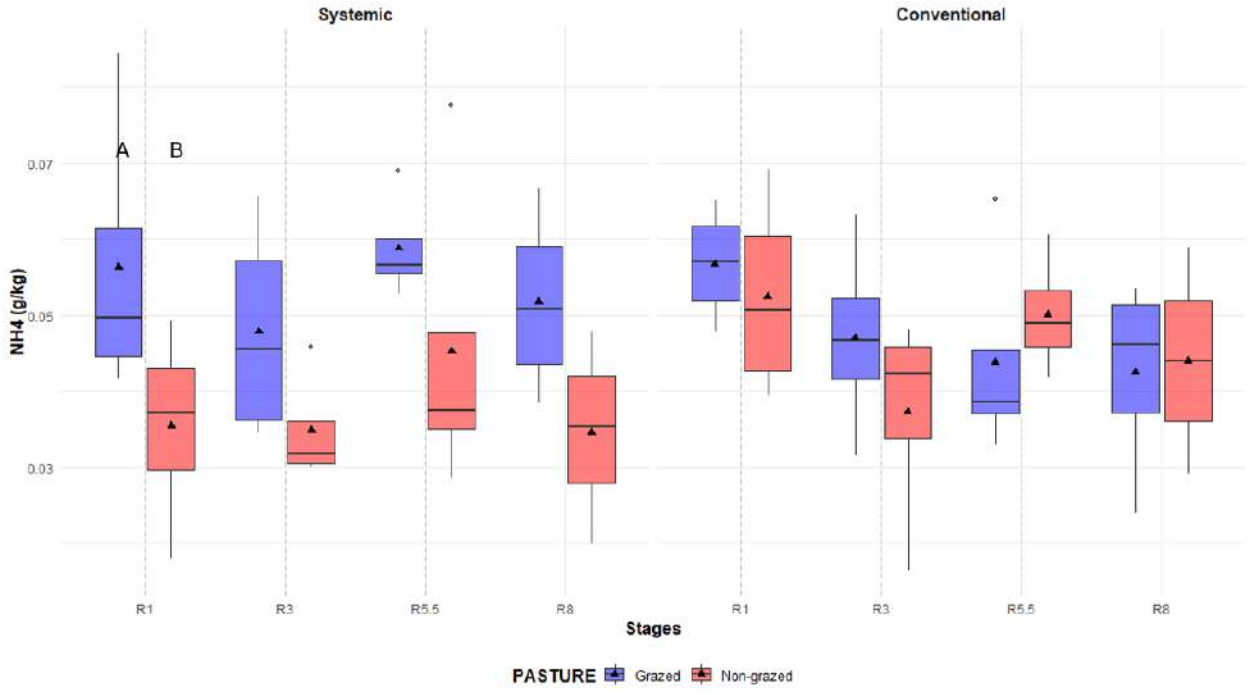


Figure 24: Boxplot of the evolution of NH4 in the soil (g/kg) during the 4 collect days by factor combination with the representation of the mean (△), the significant difference of multiple comparison test between grazing modalities is represented by the letters "A" and "B".

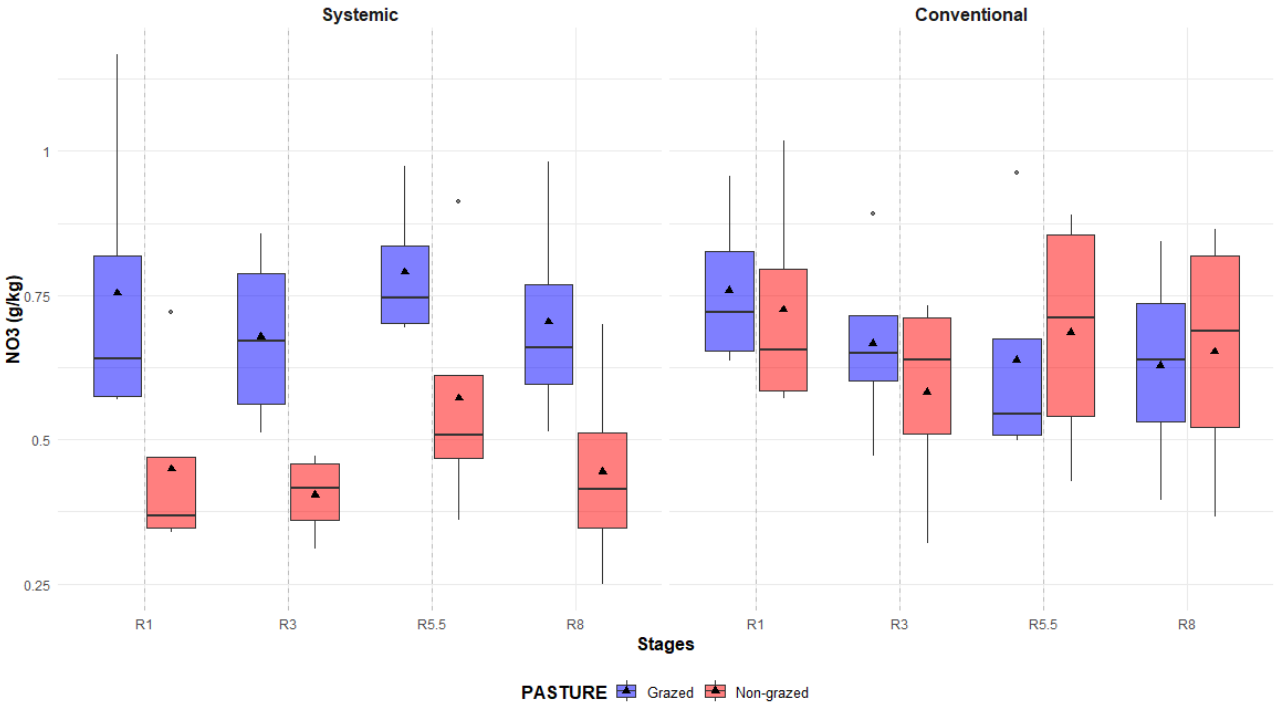


Figure 25: Boxplot of the evolution of NO3 in the soil (g/kg) during the 4 collect days by factor combination with the representation of the mean (△).

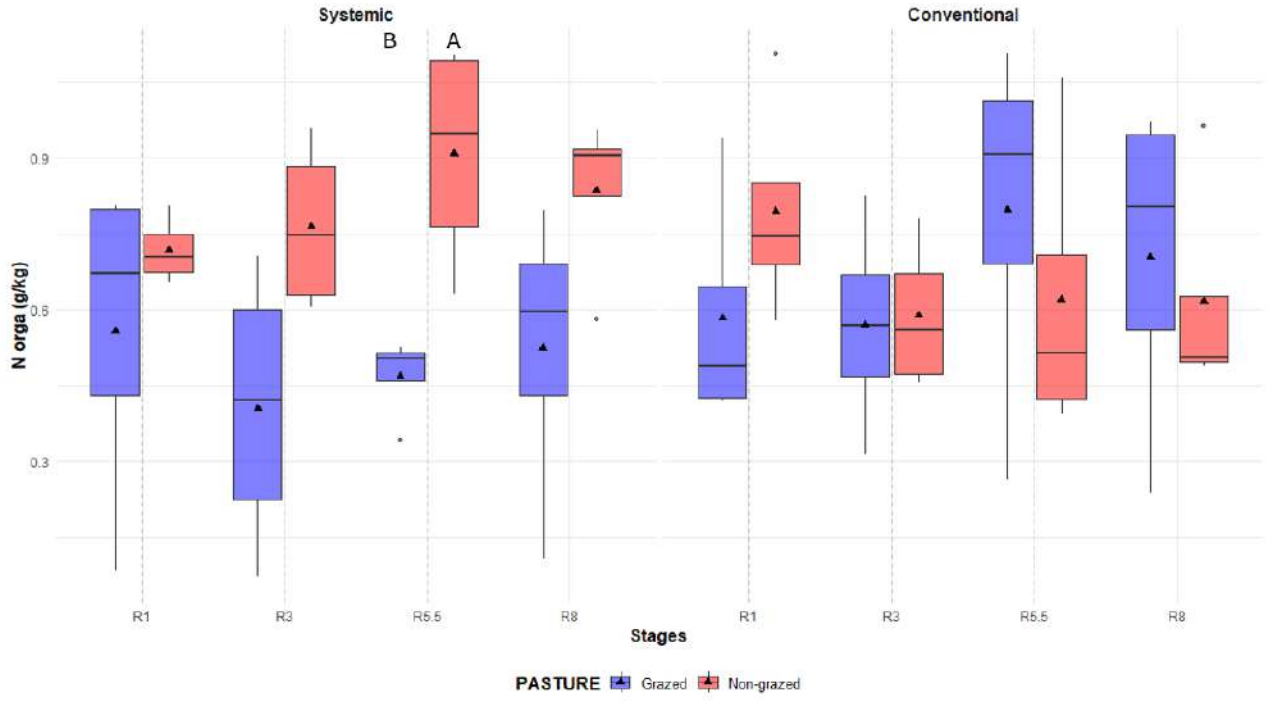


Figure 26: Boxplot of the evolution of N orga in the soil (g/kg) during the 4 collect days by factor combination with the representation of the mean (△), the significant difference of multiple comparison test between grazing modalities is represented by the letters "A" and "B".

IV Correlations

The significant correlations of interest from the divers GGpairs have been included in this section. After a general study of correlations, matrices were generated for each combination of factors in order to highlight the significant effects and compare them with the univariate analyses (*Figure 37, Figure 38, Figure 40, Figure 39, Figure 41*). Pearson tests and regression graphs were conducted to observe potential correlations between variables under the different constraints of the experimental setup.

IV.1 Nitrogen in the plant and total nitrogen in the soil

The results of the correlation, between the nitrogen rate variable in the aerial tissues and the total nitrogen in the soil at flowering stage (R1) are to follow. Firstly, *Figure 27* indicates a positive correlation and minimal dispersion between nitrogen rate variable in the aerial tissues and the total nitrogen in the soil at flowering stage (R1) consideration in the grazed conditions (*Table 5.6*). The Pearson test provides confirmation of a positive correlation between the two variables in the two grazed conditions. Based on these results, there is sufficient evidence to assert the existence of a linear relationship between the variables in these groups.

In the case of the non-grazed modalities, a considerable degree of dispersion was observed, accompanied by a negative correlation between the two variables in the context of conventional fertilisation. In the case of the non-grazed-systemic modality, the graph demonstrates a lack of correlation between the variables, as evidenced by the coefficient approaching 0 of the *table 27*. Nevertheless, the high p-value precludes the establishment of a correlation between variables for the non-grazed modalities. The inclusion of confidence intervals that encompass zero serves to reinforce the notion that no significant correlation exists.

Table 5.6: Table of Pearson correlation coefficients between nitrogen levels in the plant and total soil nitrogen at the R1 stage by combination of factors.

R1	PASTURE	FERTI	estimate	statistic	p.value	conf.low	conf.high
1	Grazed	Systemic	0.877	5.78	$1.77 * 10^{-4}$***	0.611	0.965
2	Grazed	Conventional	0.964	11.4	$4.6 * 10^{-7}$***	0.873	0.990
3	Non-grazed	Systemic	0.00493	0.0156	0.988	-0.571	0.577
4	Non-grazed	Conventional	-0.331	-1.11	0.293	-0.761	0.300

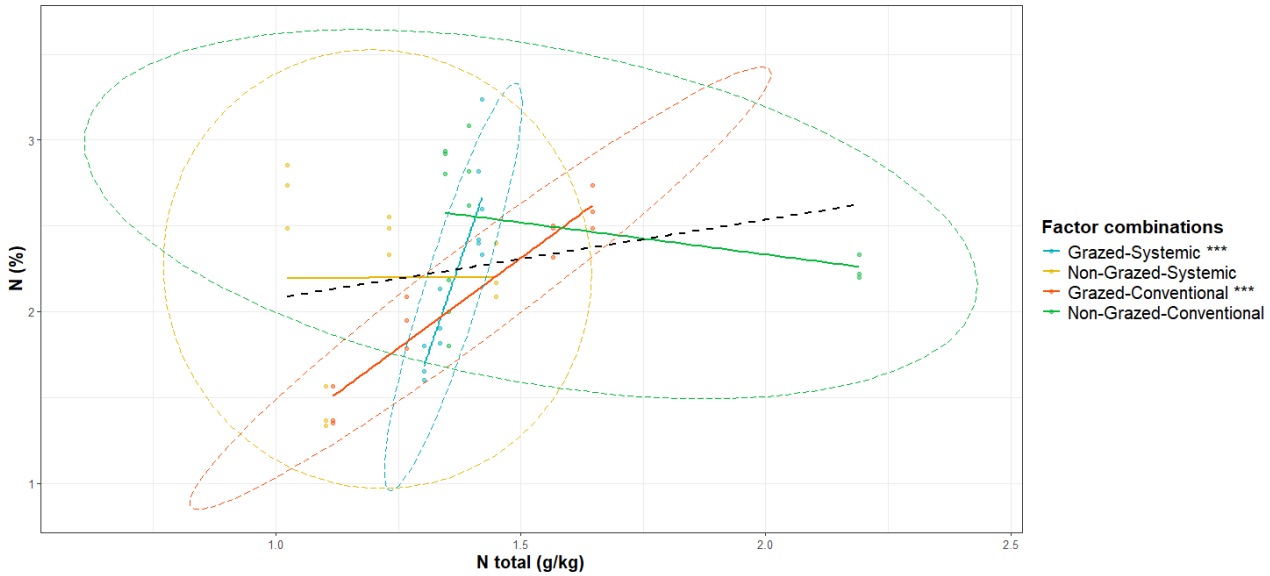


Figure 27: Regression graph between N total of the soil and N content in the plant at R1 stage and their significant correlation (p-value<0.001= ***) for each factor combinations with the global regression line in dotted black and the dispersion in dotted colour.

IV.2 Nitrogen in the plant

In *Table 5.7*, the p-value<0.05 of the Pearson test indicates a significant negative correlation between the nitrogen quantity in the plant at flowering and the pod nitrogen quantity at maturity for the non-grazed-conventional modality (*Figure 28*). The amount of phosphorus and potassium in the plant at the R1 stage was also compared with the amount of nitrogen in the pods at maturity, and a negative correlation was also found between these variables (p-value near to significance) (*Table B.7*). The nutrients present in the tissues at the flowering stage would therefore have an inverse tendency with the nitrogen present in the pods.

Table 5.7: Table of Pearson correlation coefficients between plant nitrogen quantity at R1 and pod nitrogen quantity at the R8 stage by combination of factors.

	PASTURE	FERTI	estimate	statistic	p.value	conf.low	conf.high
1	Grazed	Systemic	-0.458	-1.63	0.135	-0.817	0.158
2	Grazed	Conventional	-0.196	-0.631	0.542	-0.692	0.426
3	Non-grazed	Systemic	0.300	0.993	0.344	-0.331	0.745
4	Non-grazed	Conventional	-0.606	-2.41	0.037*	-0.875	-0.0488

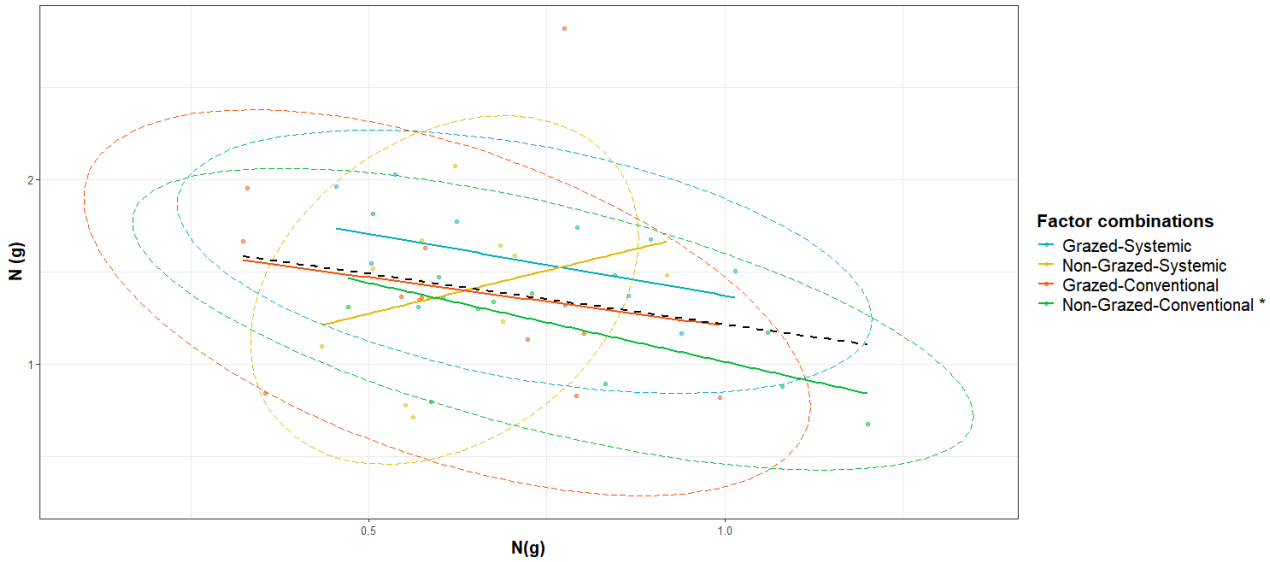


Figure 28: Regression graph between the plant nitrogen at R1 stage and pod nitrogen at R8 stage and their significant correlation (p-value<0.05 = *) for each factor combination with the overall regression line in dotted black and the dispersion in dotted colour.

IV.3 Pod nitrogen content and pod ratio

The p-value<0.05 of the grazed-systemic modality indicates a significant positive correlation as illustrated in the estimate column of *Table 5.8* and *Figure 29*.

Table 5.8: Table of Pearson correlation coefficients between pod nitrogen levels and biomass/pod at the R5.5 stage by combination of factors.

	PASTURE	FERTI	estimate	statistic	p.value	conf.low	conf.high
1	Grazed	Systemic	0.588	2.30	0.0445*	0.0206	0.869
2	Grazed	Conventional	0.130	0.414	0.687	-0.480	0.655
3	Non-grazed	Systemic	0.204	0.659	0.525	-0.419	0.696
4	Non-grazed	Conventional	0.365	1.24	0.243	-0.264	0.776

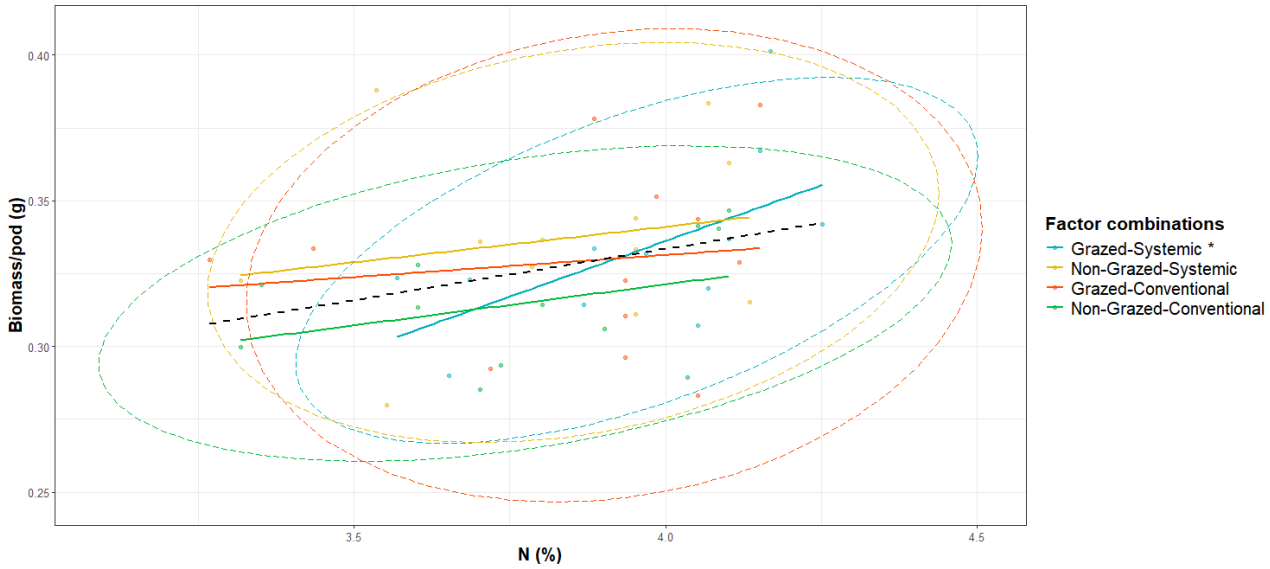


Figure 29: Regression graph between the pod nitrogen and biomass/pod at R5.5 stage and their significant correlation (p-value<0.05= *) for each factor combination and with the global regression line in dotted black and the dispersion in dotted colour.

IV.4 Photosynthesis and nitrogen quantity in the plant

Table 5.9 shows a high significance (p-value<0.01) in the positive correlation for the grazed-systemic combination. The relation is clearly observable in *Figure 30* .

Table 5.9: Table of Pearson correlation coefficients between net photosynthetic rate at R6 stage and plant nitrogen at R8 stage by combination of factors.

	PASTURE	FERTI	estimate	statistic	p.value	conf.low	conf.high
1	Grazed	Systemic	0.709	3.18	0.009**	0.229	0.912
2	Grazed	Conventional	-0.0524	-0.166	0.872	-0.608	0.538
3	Non-grazed	Systemic	0.0657	0.208	0.839	-0.528	0.616
4	Non-grazed	Conventional	-0.436	-1.53	0.156	-0.808	0.184

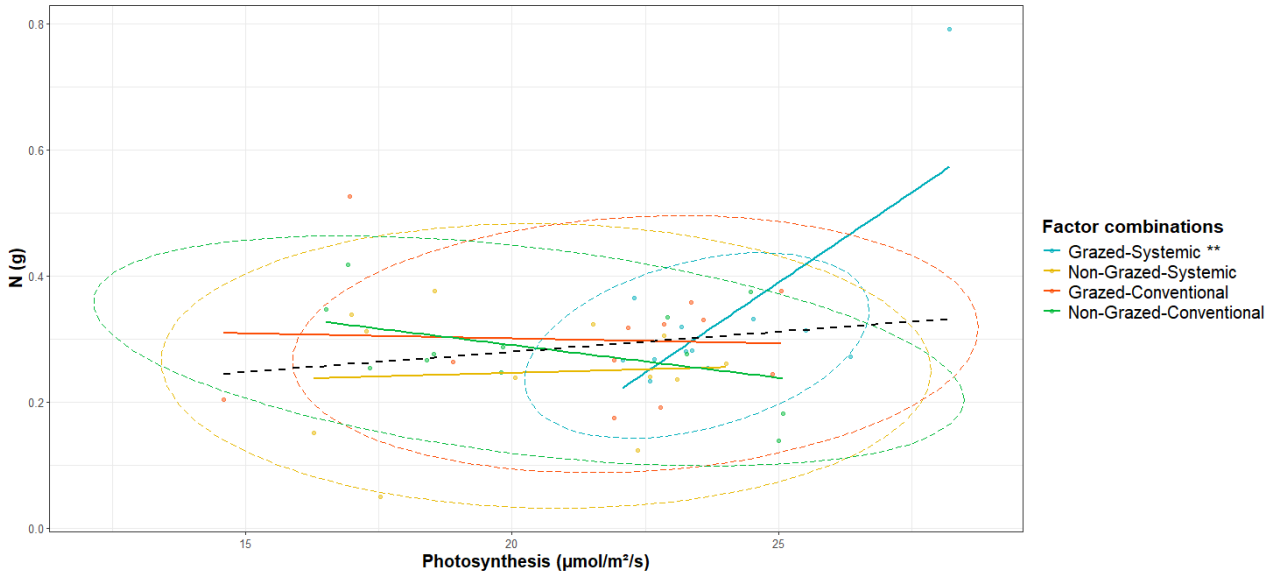


Figure 30: Regression graph between the photosynthetic rate at R6 stage and plant nitrogen content at R8 stage and their significant correlation (p-value<0.01= **) for each factor combination and with the global regression line in dotted black and the dispersion in dotted colour.

V Linear discriminant analysis

This section contains the results of a discriminant analysis carried out on 20 variables of interest from the different cycles harvested. The Wilk's lambda test revealed a p-value $< 2.2^{-16}$ for the factor combinations. Therefore, those have a highly significant effect on the 3 LDA discriminants.

Table 5.10: Variables used in linear discriminant analysis.

Variable	Description	Variable	Description
LAI_R5.5	LAI at R5.5 stage	Photo_R6_5D	Net photosynthetic rate at R6 stage, 5 days later (µmol CO2/m²/s)
LAI_R6	LAI at R6 stage	Photo_R7	Net photosynthetic rate at R7 stage (µmol CO2/m²/s)
LAI_R6_5D	LAI at R6 stage, 5 days later (g)	Biomass/pod R5.5	Biomass per pod at R8 stage (g)
N veg tissue R1	N quantity in vegetative tissue at R1 stage (g)	Biomass/pod R8	Biomass per pod at R8 stage (g)
N veg tissue R5.5	N quantity in vegetative tissue at R5.5 stage (g)	Productivity	Yield (kg/ha)
N veg tissue R8	N quantity in vegetative tissue at R8 stage (g)	Root biomass R1	Root biomass at R1 stage (g)
N pod R5.5	N quantity in pods at R5.5 stage (g)	TGW	Thousand grain weight (g)
N pod R8	N quantity in pods at R8 stage (g)	Veg biomass R1	Biomass of vegetative tissue at R1 stage (g)
Photo_R5.4	Net photosynthetic rate at R5.4 stage (g)	Veg biomass R5.5	Biomass of vegetative tissue at R5.5 stage (g)
Photo_R6	Net photosynthetic rate at R6 stage (g)	Veg biomass R8	Biomass of vegetative tissue at R5.5 stage (g)

Figure 31 exposes that the LD1 discriminant provides good separation between the 2 grazed and non-grazed groups. This discriminant corresponds to the first linear combination of variables that maximises the separation between classes while minimising the variance within classes. In this study, the classes represent combinations of factors as shown in *Figure 33*. Moreover, discriminant 2 separates the Systemic and Conventional groups under the non-grazed effect. Whereas, in the grazed treatments, on the left of the graph, the ellipses merge more closely. *Figure 32* reveals a better separation between the Grazed-systemic and Grazed-conventional groups for the discriminant 3.

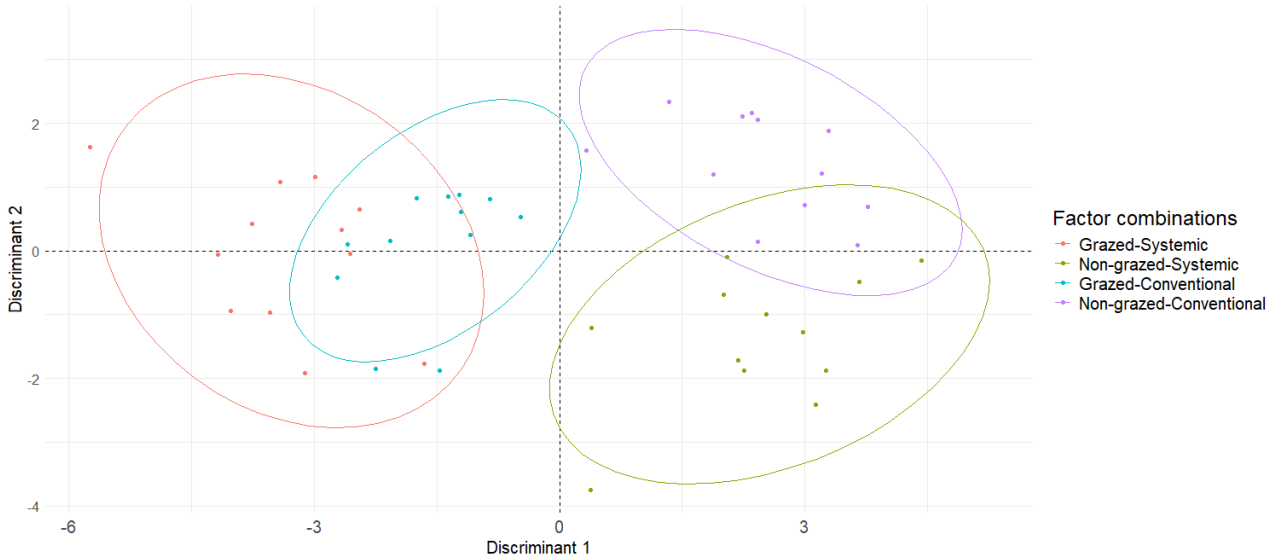


Figure 31: LDA between discriminant 1 and 2 of interest variables with ellipses per factor combinations.

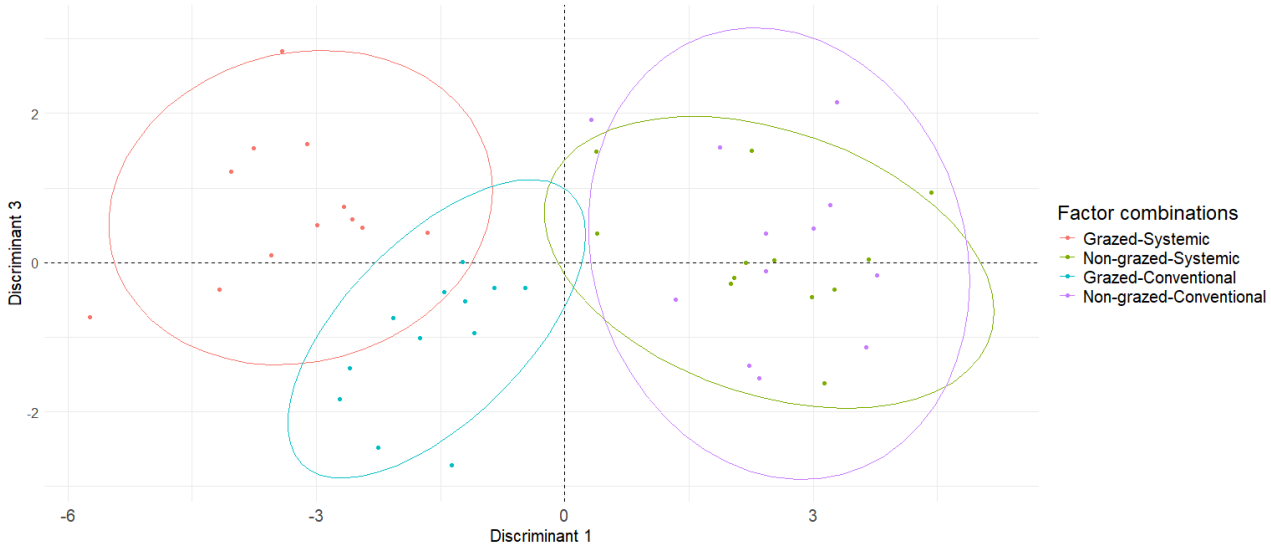


Figure 32: LDA between discriminant 1 and 3 of interest variables with ellipses per factor combinations.

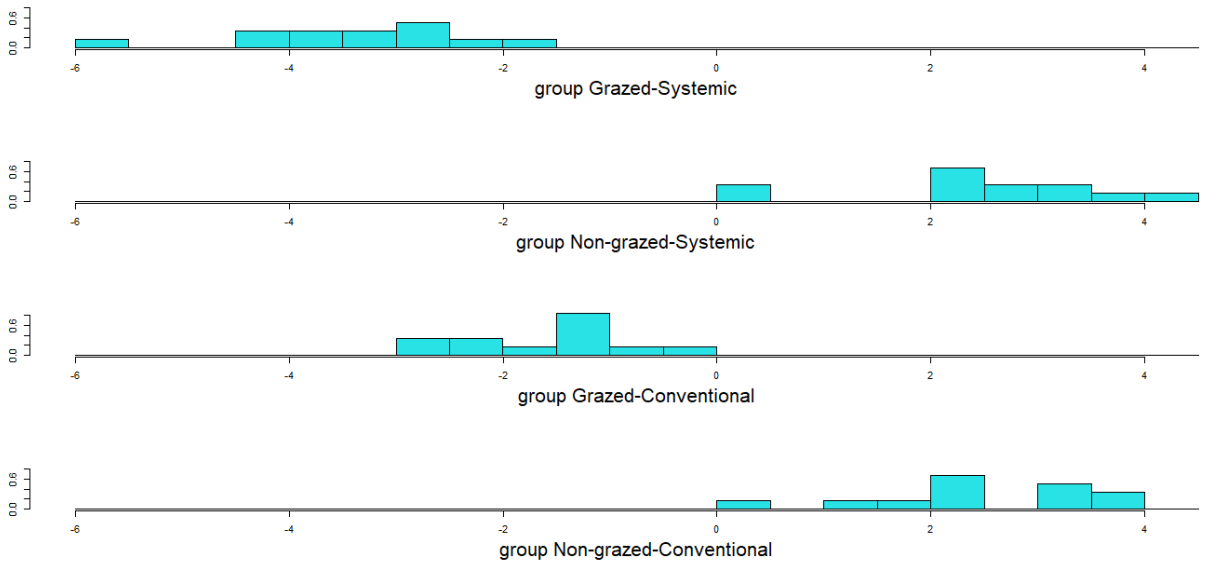


Figure 33: Histogram of the population for each combination of factors.

Although having opposite sign coefficients, biomass/pod at R5.5 and R8 stage have a strong contribution for discriminant 1. LAI R6 and LAI R6 + 5 days and N veg R8 have a significant negative contribution for this discriminant as well (*Figure 34*). The coefficients of strong contributions for discriminant 2 are N veg at each stage, N pod as well as the biomass/pod at the two stages collected. All these variables have negative coefficients except for N veg at the R1 stage. For more details, the relative contribution levels of each explanatory variable to class separation in the LDA model is exposed in *Table B.8*.

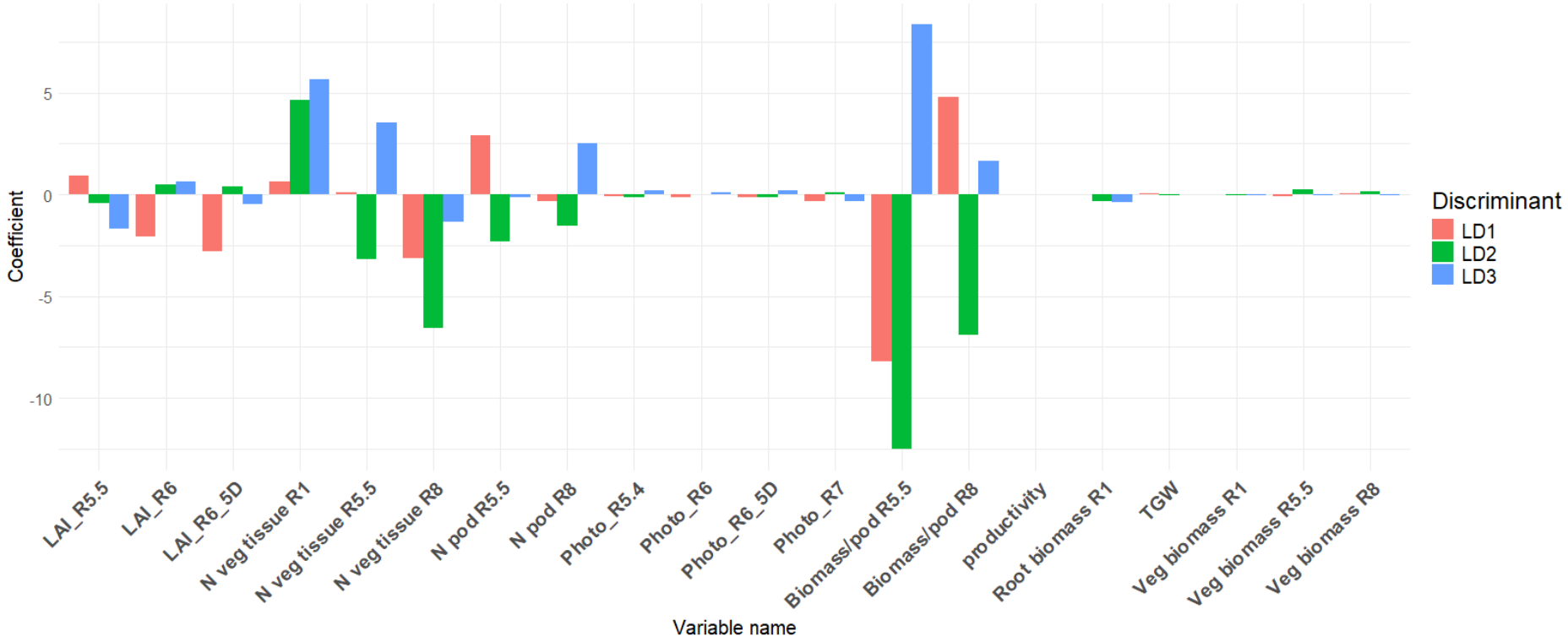


Figure 34: Variable contribution to discriminants.

Chapter 6

Discussion

The significant effect of grazing on soil mineral nitrogen in the topsoil during the flowering stage indicates more efficient mineralisation in integrated crop-livestock systems (ICLS). The 0-10 cm soil layer in grazed plots generally contains higher levels of NO3 and NH4 compared to non-grazed plots at this stage. In ICLS, sheep return most of the nutrients they consume through their excreta, resulting in an enhanced nutrient cycling process (Alves et al., 2022). Post-grazing, the rumen processing of cover crops contributes to a faster decomposition of plant material than microbial decomposition in the soil. ICLS foster an environment more conducive to soil microorganism coexistence compared to traditional cropping systems (de Moraes et al., 2013). The efficiency of nutrient recycling through grazing cover crops can reach up to 75% (Li et al., 2021). Consequently, nutrients become more readily available to the subsequent crop (Cicek et al., 2014). Conversely, non-grazed plots that receive systematic fertilisation exhibit significantly higher levels of organic nitrogen in the top 10 cm of soil at flowering stage (*Figure 16*). This may be due to a denser vegetation cover. Santos et al. (2019) demonstrated that the implementation of grazing practices led to an enhancement in the quality of the above-ground litter of cover crops, consequently resulting in a more expeditious rate of decomposition when compared to non-grazed plots. Moreover, Italian ryegrass, benefiting from potassium and phosphorus inputs from systemic fertilisation, would produce more biomass, which, not being consumed by ruminants, takes longer to mineralise.

Nitrogen is evenly distributed between sheep faeces (20-55%) and urine (45-50%). Phosphorus is mostly excreted through faeces (>95%), while potassium is predominantly excreted in urine (70-90%). Grazing increases the mineralisation of nitrogen and phosphorus, as well as their availability in the soil (Shand & Coutts, 2006). Ghimire et al. (2021) found that integrating animals into the agroecosystem reduces the crop's dependency on chemical fertilisers and enhances nitrogen use efficiency. In the current study, the abundance of mineral nitrogen in the first 10 cm in ICLS can be linked to the correlation between plant nitrogen content and total soil nitrogen at flowering. However, despite the significance of grazing in the ANOVA, the multiple comparison test only presents a significant difference for NH4 in the top 10 cm of soil. This may be due to the limited data on soil nitrogen analysis (4 per treatment), resulting in a loss of power in the multiple comparison test. As noted by Lee & Lee (2018), multiple comparison tests of Tukey may lack sufficient power to detect specific differences between treatment pairs. Moreover, the conditions of the previous study, which ran from 2003 to 2017, likely influenced the results of the current experiment as well. Soil measurements were taken in the top 10 cm, so potential nutrient leaching over the years could not be identified. Focusing solely on the top 10 cm also limits the interpretation of soil health. Nitrogen distribution is not uniform within the

soil, and its physico-chemical properties vary with depth. Significant amounts of nitrogen can be found in deeper horizons (10-30 cm and beyond), especially in well-drained soils where water infiltration and nutrient leaching are common, particularly for nitrate. Ignoring these horizons may underestimate the total nitrogen availability to the plant (Riley et al., 2001). Even if the majority of the root biomass is located between 15 and 30 cm deep, soybean roots can often reach depths of 1 meter or more (Greg Endres, NDSU Extension cropping systems specialist & Hans Kandel, NDSU Extension agronomist, 2021). Roots extract nutrients and water from deeper layers, so limiting the study to the surface layer does not fully reflect the reality of root nutrient uptake. Soils are heterogeneous, and physicochemical properties vary with depth. A study confined to the top 10 cm may not capture this variability, leading to an incomplete understanding of nitrogen availability and dynamics in the soil profile. Despite these limitations, ammonium levels in the studied horizon of systematically fertilised plots were higher in ICLS compared to non-grazed plots (*Figure 13*), indicating more plant-assimilable mineral nitrogen at this stage. However, soybean plants only assimilate about 20% of total nitrogen until the initial flowering stage (R1) (Ohyama et al., 2017). It is, therefore, crucial not to draw premature conclusions regarding the cycle of the plant. It is possible that ICLS plants under systematic fertilisation may exhibit poorer nitrogen assimilation and fixation later in their cycle. However, the results in *Table 5.3* show a significant impact of grazing, with systematically fertilized grazed plots having significantly more nitrogen in their vegetative tissues at maturity. The increased nitrogen content in plants under these conditions allows us to hypothesize efficient nitrogen assimilation throughout the cycle (*Figure 23*) and good translocation to pods (*Figure 22*).

The systemic fertilisation approach, where phosphorus (P) and potassium (K) are applied along with nitrogen (N), can enhance root development and nutrient uptake efficiency, thereby improving the soybean plant's ability to effectively assimilate soil nitrogen during critical growth periods. Moreover, this practice can enhance biological nitrogen fixation by creating a nutrient-rich environment for nitrogen-fixing bacteria in root nodules (Chaudhary et al., 2008). It is important to note that nitrogen in pods was analyzed in this study, not nitrogen in grains. Therefore, it does not fully reflect the nitrogen exported by soybean and its contribution to crop yield.

Given that P and K exports through meat are minimal (Alves et al., 2019), nutrient availability in soils receiving systematic fertilisation is expected to be sufficient for soybean production after grazing. Consequently, this system-focused strategy, based on periods of high and low nutrient exports, can also improve P and K use efficiency (Alves et al., 2022). However, this study shows lower potassium concentrations in vegetative tissues of systematically fertilised plants at maturity (*Figure 18*). Two hypotheses emerge. The first one concerns the interaction between potassium and the soil. As previously explained, potassium is primarily returned through sheep urine. In urine, potassium exists in ionic form ($K+$) and is highly soluble in water. According to Alves et al. (2019), potassium accumulates in an adsorbed and non-exchangeable form on the 2:1 clay interlayer, with some potentially migrating to lower soil layers, rendering it unavailable to soybean plants because it is beyond the reach of their roots. The 2:1 clay layer consists of an octahedral sheet sandwiched between two tetrahedral sheets and is the most common clay mineral family encountered in sediments. The second hypothesis concerns the dynamics of potassium in plant tissues. Low potassium content in soybean tissues in ICLS under systematic fertilisation could be due to potassium translocation to the pods. During plant growth, particularly in reproductive stages, potassium is translocated from older to younger tissues and

from vegetative parts to developing pods and seeds. In the case of potassium deficiency in the upper soil horizons, initial potassium levels may reach insufficient levels, and redistribution may deplete vegetative tissues of K as the plant prioritizes reproductive tissues. In addition, despite the significant effect of grazing on the amount of phosphorus in the plant, no difference in means was found between treatments. As explained above, this could be due to lack of power in the post-hoc tests. Nevertheless, the fertilisation history of the experimental field led to P saturation at the different sites. This reduced the immobilisation capacity of the soil and converted the phosphorus provided by the different treatments into less available forms (Alves et al., 2019). This phenomenon could have limited the effects of different treatments on this variable.

However, no significant treatment differences were observed for nitrogen and potassium quantities in grams at maturity, despite significant percentage differences. This result could be due to a dilution effect of nutrient concentration in the tissues. The nutrient percentage measured is a relative value, indicating the amount per unit mass of tissue, independent of the total biomass. Having a lower potassium concentration but higher total biomass leads to a greater absolute quantity in the plant. This could explain why grazing is no longer significant for nitrogen and potassium quantities in soybean plants (*Table 5.3*).

Figure 17 shows that plants in these conditions have more pods and higher pod biomass. According to Singh et al. (2022), the number of pods produced is contingent upon the number of flowers and the rate of pod formation. Consequently, environmental conditions during the flowering and pod formation periods are inextricably linked to the number of pods produced. The present study revealed that the top 10 cm of soil at the flowering stage exhibited elevated levels of mineral nitrogen in ICLS with systemic fertilisation. The favourable conditions resulted in an increase in the number of pods at the maturity stage. A correlation was found between nitrogen in pods and pod biomass at the late filling stage in ICLS plots with systematic fertilisation. Indeed, plants at maturity under this condition have pods that are on average 19% larger than those under other conditions. Despite the lack of statistical significance for pod nitrogen content at maturity, the nitrogen dynamics in the plant analysed above suggest that soybean plants in ICLS with systematic fertilisation tend to produce more pods with higher nitrogen levels.

Other physiological parameters showed differences, such as the LAI and photosynthesis rate from the full grain stage (R6). The difference between ICLS and non-grazed plots increases throughout the soybean cycle. Indeed, the LAI of plants in this system declines less steeply than that of non-grazed plots (*Figure 20*). However, the LAI 2000 Plant Canopy Analyzer is among the devices measuring gap fraction. The gap fraction approach does not distinguish photosynthetically active leaf tissue from other plant elements, such as stems, branches, or flowers. The conventional inversion models used by this instrument cannot measure the area contributed by green leaves only and compensate for the non-random positioning of canopy elements. In discontinuous and heterogeneous canopies, like in the present study, the potential of this tool is limited by a general tendency to underestimate LAI. It is therefore important to qualify the results obtained with this instrument (Jonckheere et al., 2004). It is also important to note that higher LAI does not necessarily lead to higher yields. In their study, He & Matthews (2023) found that a soybean canopy with a lower LAI resulted in higher yields. This is thought to be due to a change in light use in the vertical canopy profile where a less dense canopy reduces shading of the upper layers, increasing the light incident on the lower leaves.

Nevertheless, in the current study, higher photosynthesis rates from stage R6 onwards for ICLS receiving systemic fertilisation are observed (*Figure 21*). Kaschuk et al. (2010) found that soybean plants with higher rates of photosynthesis delayed senescence. This is strongly linked to nodulation activity and highlights two processes. The first is the fixation of N2 by the nodules, which delays the remobilisation of nitrogen from vegetative parts to the seeds. The second process involves the stimulation of leaf activity by carbon sinks, leading to increased photosynthetic activity. These processes can be correlated with the current study's results, which show significantly higher nitrogen levels in the vegetative parts of plants receiving systematic fertilisation in the ICLS plots at maturity stage. This hypothesis is supported by the relationship observed between the rate of photosynthesis at the R6 stage and plant nitrogen at the R8 stage in the grazed-systemic fertilisation combination (*Figure 30*). Under these conditions, the significant increase in net photosynthesis rate results to the accumulation of nitrogen in their leaves. The delay in leaf senescence therefore increases grain filling (Kaschuk et al., 2010). In the current study, no difference between the treatments was observed in grain yield. The biomass of pods and their number had to be observed to see an advantage of grazing in the systemically fertilised plots.

According to Gonçalves e Silva et al. (2024), soil organic matter content and total carbon content greatly increased under integrated crop-livestock systems because of the higher addition of carbon through the decomposition of soil cover promoted by these cultivation systems. In addition, soil organic matter and total organic carbon content show a significant positive correlation with the photosynthetic rate and aboveground biomass production of soybean (Gonçalves e Silva et al., 2024). The best soil conditions in integrated crop-livestock systems would therefore improve the physiology and biomass accumulation of soybean. Even if in the current study, no soil carbon analysis was carried out, it can be observed that grazing has a positive effect on plant biomass and that, for ICLS with systemic fertilisation, the total biomass at maturity is significantly higher (*Figure 17*). The nutritional advantages of systemic fertilisation mentioned earlier would accentuate the added value of grazing on plant biomass accumulation. In his study, Makino (2011) noted that the absorption capacity of rice plants limits yield potential. This means that improving sink capacity is no longer effective and that improving photosynthesis and biomass production is the only remaining goal for any further increase in the yield potential. However, this phenomenon cannot yet be linked to soybean cultivation.

During this work, results from linear discriminant analysis led to the conclusion that productivity does not influence the separation of different treatment groups. As a reminder, the yield variable does not distinguish between non-grazed and grazed plots or plots receiving systematic or conventional fertilisation. Furthermore, univariate analyses on this variable suggest no significant difference between the different treatments, although a slightly higher average (almost 500 kg/ha) was observed between ICLS with systematic fertilisation and non-grazed plots with conventional fertilisation. As Alves et al. (2022) confirms, there is no reduction in soybean productivity in plots where animals have been introduced. This is an important result because soybean is a summer crop that requires high soil fertility. This indicates that the fertiliser applied during the grazing phase was retained in the soil and readily taken up by soybean plants (Farias et al., 2020). There are several possible explanations for the lack of difference in productivity between treatments, despite the variations observed previously. Firstly, it is important to point out that prior to the yield methodology, the biochemical and structural

composition parameters of all the pods were measured and not just the seeds. Indeed, the pod hull was also part of the sample for ease of handling when monitoring the soybean cycle probably played a role in the significant effects of the treatments observed. The differences observed above are therefore not necessarily linked to the yield results, and may also be due to an incorrect estimate of the number of plants per hectare. As a reminder, the yield calculation methodology entailed the collection of six random samples per plot. For each sample, the plants along a 2-metre row were collected and counted. Subsequently, the number of plants was extrapolated in order to estimate the number of plants per hectare. However, this extrapolation does not necessarily reflect the actual density of the plants, which could have masked the differences in yield between the treatments. Finally, it is also possible that stress factors may have contributed to an alteration in the potential quality of the yield. A lack or excess of water or extreme temperatures can disrupt certain metabolisms and damage cell structures. For example, Staniak et al. (2023) have demonstrated that abiotic stresses release reactive oxygen species (ROS), which are by-products of altered aerobic metabolism. At high concentrations, these compounds can damage cells and affect plant yields.

Contribution

This master's thesis was born out of a personal desire to work on an agroecological project abroad. This desire to learn how agronomic research works in another part of the world had been with the student for a long time. Following numerous meetings with his two promoters, Jérôme Bindelle and Paulo César de Faccio Carvalho, he had the chance to choose to work on integrated crop-livestock systems in Rio Grande do Sul in southern Brazil. He spent 3 months at the experimental station of the agronomic faculty of the Federal University of Rio Grande do Sul, and his experimentation was part of the research group on grazing ecology : Grupo de Pesquisa em Ecologia do Pastejo (GPEP). Throughout the entirety of the student's stay, he engaged with the objective of integrating into the group, acquiring new knowledge, and learning the national language, Portuguese. In exchange, he was able to receive help from students with his master thesis.

This work required a great deal of adaptation to the terrain. Indeed, one of the first constraints was the language barrier. Although some of the university staff spoke English, most of the people the student worked with did not. Therefore, he had to learn the rudiments of Portuguese to facilitate his work and his integration. The meteorological conditions and access to equipment meant that he had to improvise when taking data on certain parameters such as LAI and photosynthetic rate. The choice of this master's thesis represented a risk because of the indeterminacy of harvest dates due to the instability of the evolution of the soybean cycle. In addition, personal decisions to limit the number of samples were taken to favour rapid and inexpensive laboratory analysis.

Chapter 7

Conclusion and perspectives

The principal objective of this study is to examine the nutrient dynamics and biomass production of soybean in no-till integrated crop-livestock systems under systemic fertilisation in the southern region of Brazil. The integration of ICLS and systemic fertilisation would led to an enhancement in soil nutrient availability. This resulted in the plant assimilating these elements with greater efficiency during its reproductive growth cycle, leading to an increase in biomass production compared to non-grazed systems. The favourable nutritional conditions in the soil led to an increase in the photosynthetic parameters of ICLS soybean plants, which exhibited a slower decline at the end of the cycle. However, no difference in yield between treatments was observed. This may be explained by the limitations of the methodology, differences in sampling or the possible influence of abiotic factors. ICLS combined with systemic fertilisation is therefore a potential way to increase food production and improve the sustainability and productivity of agro-ecosystems. In view of the findings of the present study, there are a number of avenues for further research.

In this study, the analyses focused on the top 10 cm of soil. A relevant perspective would be to deepen these analyses by examining the deeper soil horizons (10-20 cm and beyond). This would make it possible to identify any leaching of nutrients and obtain a more complete picture of the distribution of nitrogen and other nutrients. As mentioned in the study, soil organic carbon was not analysed. Analysing the impact of grazing practices on the quality of soybean beans (protein content, oil content, etc.), in order to understand whether these practices influence not only the quantity but also the quality of production, would be beneficial for the farming community. As this study took place during part of the soybean cycle, it would also be interesting to analyse the biomass and nutrients present in Italian ryegrass (*Lolium multiflorum* Lam.) crop in order to gain a better understanding of soil-plant interactions in ICLS as a function of the type of fertilisation. In order to gain a broader view of the potential benefits of ICLS and systemic fertilisation, the development of predictive models could be explored. Modelling nutrient dynamics in ICLS systems could be used to predict the long-term results of grazing and fertilisation practices. This could include simulations for different climatic conditions and land use scenarios. Finally, analysing the efficiency of integrated crop-livestock systems under different fertilisation techniques in other soil and climatic conditions would enable us to broaden the study and attempt to propose alternatives that are better suited to specific regions. More in-depth studies would give farmers a better idea of what ICLS are and how, with careful management and inputs, they can be a means of sustainable intensification and provide a partial response to land-use issues.

Bibliography

Alvares C.A. et al., 2013. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*:711–728. doi:10.1127/0941-2948/2013/0507. Publisher: Schweizerbart'sche Verlagsbuchhandlung.

Alves B.J.R., Boddey R.M. & Urquiaga S., 2003. The success of BNF in soybean in Brazil. *Plant and Soil*, 252(1):1–9. doi:10.1023/A:1024191913296.

Alves L.A. et al., 2019. Soil acidification and P, K, Ca and Mg budget as affected by sheep grazing and crop rotation in a long-term integrated crop-livestock system in southern Brazil. *Geoderma*, 351:197–208. doi:10.1016/j.geoderma.2019.04.036.

Alves L.A. et al., 2022. Fertilization strategies and liming in no-till integrated crop–livestock systems: effects on phosphorus and potassium use efficiency. *Rev. Bras. Ciênc. Solo*, 46:–. doi:10.36783/18069657rbcs20210125. Publisher: Rev. Bras. Ciênc. Solo.

Assmann J.M. et al., 2015. Carbon and nitrogen cycling in an integrated soybean-beef cattle production system under different grazing intensities. *Pesq. agropec. bras.*, 50:967–978. doi:10.1590/S0100-204X2015001000013. Publisher: Embrapa Secretaria de Pesquisa e Desenvolvimento; Pesquisa Agropecuária Brasileira.

Babujia L.C., Hungria M., Franchini J.C. & Brookes P.C., 2010. Microbial biomass and activity at various soil depths in a Brazilian oxisol after two decades of no-tillage and conventional tillage. *Soil Biology and Biochemistry*, 42(12):2174–2181. doi:10.1016/j.soilbio.2010.08.013.

Bagale S., 2021. Nutrient Management for Soybean Crops. *International Journal of Agronomy*, 2021(1):3304634. doi:10.1155/2021/3304634.

Balbino L.C. et al., 2011. Evolução tecnológica e arranjos produtivos de sistemas de integração lavoura-pecuária-floresta no Brasil. *Pesq. agropec. bras.*, 46. doi:10.1590/S0100-204X2011001000001. Publisher: Embrapa Secretaria de Pesquisa e Desenvolvimento; Pesquisa Agropecuária Brasileira.

Carvalho P.C.d.F., 2013. Harry Stobbs Memorial Lecture: Can grazing behavior support innovations in grassland management? *Tropical Grasslands-Forrajes Tropicales*, 1(2):137–155. doi:10.17138/tgft(1)137-155. Number: 2.

Carvalho P.C.d.F. et al., 2018. Animal production and soil characteristics from integrated crop-livestock systems: toward sustainable intensification. *Journal of Animal Science*, 96(8):3513–3525. doi:10.1093/jas/sky085.

Carvalho P.C.D.F. et al., 2021. LAND-USE INTENSIFICATION TRENDS IN THE RIO DE LA PLATA REGION OF SOUTH AMERICA: TOWARD SPECIALIZATION OR RECOUPLING CROP AND LIVESTOCK PRODUCTION. *Front. Agr. Sci. Eng.*, 8(1):97–110. doi:10.15302/J-FASE-2020380. Number: 1 Publisher: Higher Education Press.

Chaudhary M.I. et al., 2008. The effect of phosphorus deficiency on nutrient uptake, nitrogen fixation and photosynthetic rate in mashbean, mungbean and soybean. *Acta Physiol Plant*, 30(4):537–544. doi:10.1007/s11738-008-0152-8.

Cicek H., Thiessen Martens J.R., Bamford K.C. & Entz M.H., 2014. Effects of grazing two green manure crop types in organic farming systems: N supply and productivity of following grain crops. *Agriculture, Ecosystems & Environment*, 190:27–36. doi:10.1016/j.agee.2013.09.028.

de Faccio Carvalho P.C. et al., 2010. Managing grazing animals to achieve nutrient cycling and soil improvement in no-till integrated systems. *Nutr Cycl Agroecosyst*, 88(2):259–273. doi:10.1007/s10705-010-9360-x.

de Moraes A. et al., 2013. Integrated crop–livestock systems in the Brazilian subtropics. *European Journal of Agronomy*, 57. doi:10.1016/j.eja.2013.10.004.

FAO R.I.P.P.a.P.D.e., 2015. World fertilizer trends and outlook to 2018. Publisher: Rome (Italy) FAO.

Farias G.D. et al., 2020. Integrated crop-livestock system with system fertilization approach improves food production and resource-use efficiency in agricultural lands. *Agron. Sustain. Dev.*, 40(6):39. doi:10.1007/s13593-020-00643-2.

Fehr W. & Caviness C., 1977. Stages of soybean development.

Ghimire S., Wang J. & Fleck J.R., 2021. Integrated Crop-Livestock Systems for Nitrogen Management: A Multi-Scale Spatial Analysis. *Animals*, 11(1):100. doi:10.3390/ani11010100. Number: 1 Publisher: Multidisciplinary Digital Publishing Institute.

Gonçalves e Silva J.A. et al., 2024. Integration crop-livestock system increases the sustainability of soybean cultivation through improved soil health and plant physiology. *Agriculture, Ecosystems & Environment*, 359:108770. doi:10.1016/j.agee.2023.108770.

Gower S.T. & Norman J.M., 1991. Rapid Estimation of Leaf Area Index in Conifer and Broad-Leaf Plantations. *Ecology*, 72(5):1896–1900. doi:10.2307/1940988. Publisher: Ecological Society of America.

Greg Endres, NDSU Extension cropping systems specialist & Hans Kandel, NDSU Extension agronomist, 2021. Soybean Growth and Management Quick Guide | NDSU Agriculture. Section: Publication.

He Y. & Matthews M.L., 2023. Seasonal climate conditions impact the effectiveness of improving photosynthesis to increase soybean yield. *Field Crops Research*, 296:108907. doi:10.1016/j.fcr.2023.108907.

Jonckheere I. et al., 2004. Review of methods for in situ leaf area index determination: Part I. Theories, sensors and hemispherical photography. *Agricultural and Forest Meteorology*, 121(1):19–35. doi:10.1016/j.agrformet.2003.08.027.

Kar S., Montague D.T. & Villanueva-Morales A., 2021. Measurement of photosynthesis in excised leaves of ornamental trees: a novel method to estimate leaf level drought tolerance and increase experimental sample size. *Trees*, 35(3):889–905. doi:10.1007/s00468-021-02088-w.

Kaschuk G. et al., 2010. Differences in photosynthetic behaviour and leaf senescence of soybean (Glycine max [L.] Merrill) dependent on N2 fixation or nitrate supply. *Plant Biology*, 12(1):60–69. doi:10.1111/j.1438-8677.2009.00211.x. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1438-8677.2009.00211.x.

Lee S. & Lee D.K., 2018. What is the proper way to apply the multiple comparison test? *Korean J Anesthesiol*, 71(5):353–360. doi:10.4097/kja.d.18.00242.

Li J., Hou F. & Ren J., 2021. Grazing Intensity Alters Leaf and Spike Photosynthesis, Transpiration, and Related Parameters of Three Grass Species on an Alpine Steppe in the Qilian Mountains. *Plants*, 10(2):294. doi:10.3390/plants10020294. Number: 2 Publisher: Multidisciplinary Digital Publishing Institute.

LI-COR, Inc., 2024. LAI-2000 | Support.

Machado L.a.Z., Balbino L.C. & Ceccon G., 2011. Integração lavoura-pecuária-floresta. 1. Estruturação dos sistemas de integração lavoura-pecuária. Accepted: 2012-05-03T11:11:11Z Publisher: Dourados: Embrapa Agropecuária Oeste, 2011.

Makino A., 2011. Photosynthesis, Grain Yield, and Nitrogen Utilization in Rice and Wheat. *Plant Physiology*, 155(1):125–129. doi:10.1104/pp.110.165076.

Marques Pires M.d.F. et al., 2023. Nutrient Uptake by Soybean Plants in Succession of Cover Crops in Northeast of Brazil. *Communications in Soil Science and Plant Analysis*, 54(7):945–963. doi:10.1080/00103624.2022.2137183. Publisher: Taylor & Francis.

Nleya T., 2019. Chapter 3: Soybean Growth Stages.

Ohyama T. et al., 2017. Role of Nitrogen on Growth and Seed Yield of Soybean and a New Fertilization Technique to Promote Nitrogen Fixation and Seed Yield. doi:10.5772/66743.

Oliveira C.A.O.d. et al., 2014. Comparison of an integrated crop–livestock system with soybean only: Economic and production responses in southern Brazil. *Renewable Agriculture and Food Systems*, 29(3):230–238. doi:10.1017/S1742170513000410.

Pagano M.C. & Miransari M., 2016. 1 - The importance of soybean production worldwide. In M. Miransari, editor, Abiotic and Biotic Stresses in Soybean Production, Academic Press, San Diego. 1–26. doi:10.1016/B978-0-12-801536-0.00001-3.

Pedrozo A. et al., 2018. Biological nitrogen fixation and agronomic features of soybean (Glycine max (L.)Merr.) crop under different doses of inoculant. *Acta Agronómica*, 67(2):297–302. doi:10.15446/acag.v67n2.56375. Publisher: Universidad Nacional de Colombia.

Peterson C.A., Deiss L. & Gaudin A.C.M., 2020. Commercial integrated crop-livestock systems achieve comparable crop yields to specialized production systems: A meta-analysis. *PLOS ONE*, 15(5):e0231840. doi:10.1371/journal.pone.0231840. Publisher: Public Library of Science.

Reichardt K. & Timm L.C., 2020. Soil, Plant and Atmosphere: Concepts, Processes and Applications. Springer International Publishing, Cham. doi:10.1007/978-3-030-19322-5.

Riley W., Ortiz-Monasterio I. & Matson P., 2001. Nitrogen leaching and soil nitrate, nitrite, and ammonium levels under irrigated wheat in Northern Mexico. *Nutrient Cycling in Agroecosystems*, 61(3):223–236. doi:10.1023/A:1013758116346.

Rodríguez-Navarro D.N., Margaret Oliver I., Albareda Contreras M. & Ruiz-Sainz J.E., 2011. Soybean interactions with soil microbes, agronomical and molecular aspects. *Agronomy Sust. Developm.*, 31(1):173–190. doi:10.1051/agro/2010023.

Sa T.M. & Israel D.W., 1991. Energy Status and Functioning of Phosphorus-Deficient Soybean Nodules 1. *Plant Physiology*, 97(3):928–935. doi:10.1104/pp.97.3.928.

Salvagiotti F. et al., 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crops Research*, 108(1):1–13. doi:10.1016/j.fcr.2008.03.001.

Santos E.R.S. et al., 2019. Sward Responses of Bahiagrass Cultivars under No Nitrogen Fertilization. *Crop Science*, 59(6):2893–2902. doi:10.2135/cropsci2019.06.0387. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.2135/cropsci2019.06.0387.

Sartor L.R. et al., 2018. Corn Yield and Grain Nutritional Status in a Crop-Livestock System with Winter/Summer Nitrogen Levels. *Int. J. Plant Prod.*, 12(4):309–314. doi:10.1007/s42106-018-0028-9.

Sekaran U. et al., 2021. Role of integrated crop-livestock systems in improving agriculture production and addressing food security – A review. *Journal of Agriculture and Food Research*, 5:100190. doi:10.1016/j.jafr.2021.100190.

Selcer P., 2015. Fabricating Unity: The FAO-UNESCO Soil Map of the World. *Historical Social Research / Historische Sozialforschung*, 40(2 (152)):174–201. Publisher: GESIS - Leibniz Institute for the Social Sciences.

Seo S.N., 2010. Is an integrated farm more resilient against climate change? A microeconometric analysis of portfolio diversification in African agriculture. *Food Policy*, 35(1):32–40. doi:10.1016/j.foodpol.2009.06.004.

Shand C.A. & Coutts G., 2006. The effects of sheep faeces on soil solution composition. *Plant Soil*, 285(1):135–148. doi:10.1007/s11104-006-0065-5.

Silva Y.F. et al., 2022. Intense Pasture Management in Brazil in an Integrated Crop-Livestock System Simulated by the DayCent Model. *Sustainability*, 14(6):3517. doi:10.3390/su14063517. Number: 6 Publisher: Multidisciplinary Digital Publishing Institute.

Singh M., Nagar S., Singh A. & Satpute G.K., 2022. Physiological Traits Based Breeding to Achieve Higher Yield in Soybean Crop. In U.C. Jha, H. Nayyar, S.K. Agrawal & K.H.M. Siddique, editors, Developing Climate Resilient Grain and Forage Legumes, Springer Nature, Singapore. 255–275. doi:10.1007/978-981-16-9848-4_12.

Souza E.D. et al., 2014. Soil quality indicators in a Rhodic Paleudult under long term tillage systems. *Soil and Tillage Research*, 139:28–36. doi:10.1016/j.still.2014.02.001.

Staniak M., Szpunar-Krok E. & Kocira A., 2023. Responses of Soybean to Selected Abiotic Stresses—Photoperiod, Temperature and Water. *Agriculture*, 13(1):146. doi:10.3390/agriculture13010146. Number: 1 Publisher: Multidisciplinary Digital Publishing Institute.

Tedesco J. M. et al, 1995. manual_rolas.pdf. Porto Alegre, RS: Universidade Federal do Rio Grande do Sul.

Tian J. et al., 2022. Phosphorus fertilization affects soybean rhizosphere phosphorus dynamics and the bacterial community in karst soils. *Plant Soil*, 475(1):137–152. doi:10.1007/s11104-020-04662-6.

Vilela L. et al., 2011. Sistemas de integração lavoura-pecuária na região do Cerrado. *Pesq. agropec. bras.*, 46:1127–1138. doi:10.1590/S0100-204X2011001000003. Publisher: Embrapa Secretaria de Pesquisa e Desenvolvimento; Pesquisa Agropecuária Brasileira.

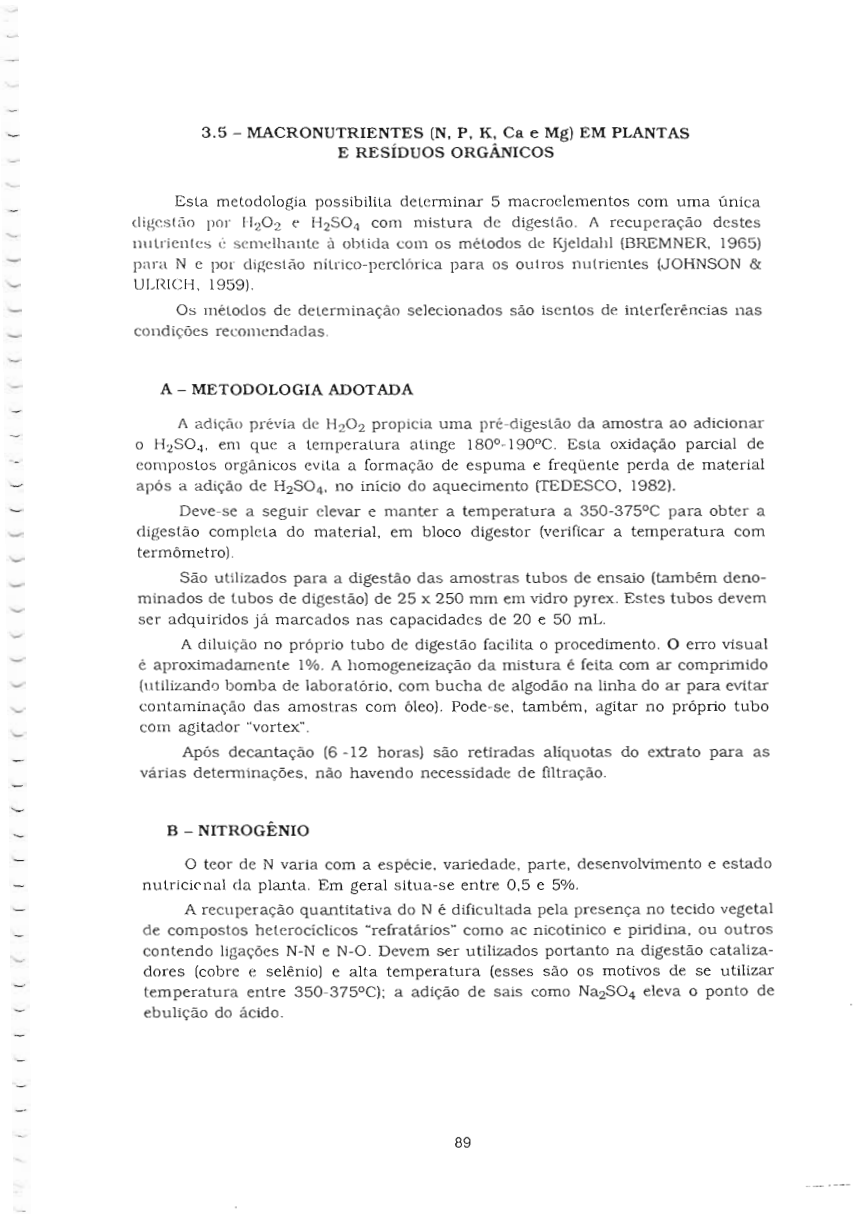
Wruck F., Behling M. & Antonio D., 2015. Sistemas Integrados em Mato Grosso e Goiás. 170–194.

Zhou M. & Yang J., 2023. Delaying or promoting? Manipulation of leaf senescence to improve crop yield and quality. *Planta*, 258(3):48. doi:10.1007/s00425-023-04204-1.

Appendix A

Laboratory protocols

I Laboratory protocols for extracting macronutrients from plant residues



3.5 – MACRONUTRIENTES (N, P, K, Ca e Mg) EM PLANTAS E RESÍDUOS ORGÂNICOS

Esta metodologia possibilita determinar 5 macroelementos com uma única digestão por H_2O_2 e H_2SO_4 com mistura de digestão. A recuperação destes nutrientes é semelhante à obtida com os métodos de Kjeldahl (BREMNER, 1965) para N e por digestão nítrico-perclórica para os outros nutrientes (JOHNSON & ULRICH, 1959).

Os métodos de determinação selecionados são isentos de interferências nas condições recomendadas.

A – METODOLOGIA ADOTADA

A adição prévia de H_2O_2 propicia uma pré-digestão da amostra ao adicionar o H_2SO_4, em que a temperatura atinge 180°-190°C. Esta oxidação parcial de compostos orgânicos evita a formação de espuma e freqüente perda de material após a adição de H_2SO_4, no início do aquecimento (TEDESCO, 1982).

Deve-se a seguir elevar e manter a temperatura a 350-375°C para obter a digestão completa do material, em bloco digestor (verificar a temperatura com termômetro).

São utilizados para a digestão das amostras tubos de ensaio (também denominados de tubos de digestão) de 25 x 250 mm em vidro pyrex. Estes tubos devem ser adquiridos já marcados nas capacidades de 20 e 50 mL.

A diluição no próprio tubo de digestão facilita o procedimento. O erro visual é aproximadamente 1%. A homogeneização da mistura é feita com ar comprimido (utilizando bomba de laboratório, com bucha de algodão na linha do ar para evitar contaminação das amostras com óleo). Pode-se, também, agitar no próprio tubo com agitador "vortex".

Após decantação (6 -12 horas) são retiradas alíquotas do extrato para as várias determinações, não havendo necessidade de filtração.

B – NITROGÊNIO

O teor de N varia com a espécie, variedade, parte, desenvolvimento e estado nutricional da planta. Em geral situa-se entre 0,5 e 5%.

A recuperação quantitativa do N é dificultada pela presença no tecido vegetal de compostos heterocíclicos "refratários" como ac nicotínico e piridina, ou outros contendo ligações N-N e N-O. Devem ser utilizados portanto na digestão catalizadores (cobre e selênio) e alta temperatura (esses são os motivos de se utilizar temperatura entre 350-375°C); a adição de sais como Na_2SO_4 eleva o ponto de ebulição do ácido.

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O procedimento de digestão adotado é baseado no método recomendado por BREMNER (1965) para solos, com inclusão da H_2O_2. As proporções de reagentes foram mantidas.

Para a determinação do NH_4^+, uma alíquota de 10-20 mL é destilada em micro-destilador descrito por BREMNER & EDWARDS (1965) modificado por TEDESCO & GIANELLO (1979), após adição de NaOH, coletando-se o destilado em indicador-ac bórico e titulando-se com H_2SO_4 diluído (ver item 2.14.1).

C – FÓSFORO

O teor de P no tecido vegetal varia em geral entre 0,08 e 1,5%.

É determinado por espectrofotometria numa alíquota do extrato após adição de molibdato de amônio e ac aminonaftolsulfônico. Este método possui sensibilidade adequada, sendo livre de interferências por H_2O_2, e sais da mistura de digestão.

D – POTÁSSIO

O teor de K no tecido vegetal varia na maior parte dos casos entre 0,2 e 10%.

É determinado por fotometria de chama após diluição do extrato, ajustando-se a sensibilidade do aparelho com os padrões adequados.

A presença de Na ($\approx$ 420 mg L^{-1}) não causa interferência, observando-se entretanto o efeito supressor de ionização (TEDESCO, 1982).

E – CÁLCIO E MAGNÉSIO

Os teores de Ca no tecido vegetal variam geralmente entre 0,05 e 2,5%, e os de Mg entre 0,02 e 1,5%.

São determinados por espectrofotometria de absorção após diluição do extrato e adição de La ou Sr em solução ácida.

3.5.1 – Material

a) Bloco digestor para tubos de digestão de 25 x 250 mm com controle eletrônico de temperatura.

b) Espectrofotômetro de absorção.

c) Fotômetro de chama.

d) Espectrofotômetro (visível).

e) Destilador de arraste de vapor (micro-Kjeldahl) conforme descrição no capítulo 2.14.1.

3.5.2 – Soluções

a) H_2SO_4 conc.

b) H_2O_2 a 30%; esta perde O_2 com o tempo, e deve ser guardada em frasco protegido da luz (pode ser usado o produto comercial de 130 vol, de baixo custo).

c) Mistura de digestão: moer 100 g de Na_2SO_4, 10 g de $CuSO_4.5H_2O$ e 1 g de selênio, e misturar bem.

d) NaOH 10M: dissolver 400 g de NaOH (produto técnico) em 800 mL de H_2O destilada em copo de becker de pyrex (ou aço inox). Após esfriar, transferir para balão vol de 1 L e completar o vol. Guardar em recipiente plástico.

e) Indicador-ac bórico: cf item 2.14.2d.

f) H_2SO_4 0,025M cf item 2.14.2e.

g) Molibdato de amônio: cf o item 2.4.2c (solução P-B).

h) Ac 1-amino-2-naftol-4-sulfônico: cf o item 2.4.2d (sol P-C).

i) Estrôncio a 0,3% em HCl 0,2M: cf item 2.7.2b.

j) Solução de 1.200 mg L^{-1} de Mg^{2+}: pesar 0,600 g de Mg metálico. Dissolver em 10 mL de HCl a 50%. Completar o vol a 500 mL com água dest.

k) Padrão misto de P, K, Ca e Mg: pesar 1,999 g de $CaCO_3$ (seco a 105°C por 2 horas) e colocar em balão vol de 1 L. Adicionar 20 mL de HCl 50% para dissolver. Adicionar 1,318 g de KH_2PO_4 e 3,472 g de KCl (secos a 105°C por 2 horas). Adicionar 200 mL da solução j (com 1.200 mg L^{-1} de Mg) e completar o vol com H_2O dest. Esta solução contém 300 mg L^{-1} de P, 2.200 mg L^{-1} de K, 800 mg L^{-1} de Ca e 240 mg L^{-1} de Mg.

3.5.3 – Procedimento

3.5.3.1 – Digestão das amostras

a) Pesar 0,200 g da amostra e colocar em tubo de digestão seco.	a) Usar funil de haste longa e Ø interno de 10 mm.
b) Adicionar 1 mL de H_2O_2.	
c) Adicionar vagarosamente 2 mL de H_2SO_4 conc (na capela).	c) A reação é rápida.
d) Adicionar 0,7 g da mistura de digestão.	d) Usar medida calib e funil de haste longa e Ø interno de 10 mm.

e) Colocar no bloco digestor a 160-180°C até evaporar a água.	e) A sol escurece devido à oxidação de compostos orgânicos solúveis não decompostos por ser baixa para evitar projeção do líquido para fora do tubo.
f) Aumentar a temperatura a 350-375°C. Após clarear (cor amarelo-esverdeada) manter esta temperatura por uma hora.	f) Ver obs 2.14.3.2.1b.
g) Retirar os frascos do bloco e deixar esfriar.	g) Colocar o conjunto numa placa de amianto ou madeira para evitar choque térmico.
h) Completar o vol com água dest até a marca de aferição (50 mL).	h) O tubo deve ter a temperatura de 50-60°C (suportável ao tato) para evitar solidificação da amostra.
i) Agitar com ar comprimido.	i) Usar um tubo capilar (ou pipeta de 1 mL) colocando um filtro de algodão no tubo flexível. Lavar e enxaguar o capilar entre amostras.
j) Transferir para frascos "snap-cap" de 90 mL. Deixar decantar algumas horas antes de retirar as alíquotas para as determinações de P, K, Ca e Mg.	Pode-se, também, deixar decantando durante a noite.

3.5.3.1.1 – Observações

a) Para as curvas padrão, medir (com microbureta) 0,0 - 0,5 - 1,0 - 2,0 - 3,5 e 5,0 mL da sol de padrão misto para tubos de digestão, seguindo o procedimento descrito a partir do item 3.5.3.1b. Após a diluição a 50 mL, ter-se-há:

mL de padrão:	0,0	0,5	1,0	2,0	3,5	5,0
teor de P (mg L^{-1}):	0,0	3,0	6,0	12,0	21,0	30,0
teor de K (mg L^{-1}):	0,0	22,0	44,0	88,0	154,0	220,0
teor de Ca (mg L^{-1}):	0,0	8,0	16,0	32,0	56,0	80,0
teor de Mg (mg L^{-1}):	0,0	2,4	4,8	9,6	16,8	24,0

Com as diluições e os métodos de determinação descritos abaixo, as concentrações finais serão:

teor de P (mg L^{-1}):	0,0	0,5	1,0	2,0	3,5	5,0
teor de K (mg L^{-1}):	0,0	2,0	4,0	8,0	14,0	20,0
teor de Ca (mg L^{-1}):	0,0	2,0	4,0	8,0	14,0	20,0
teor de Mg (mg L^{-1}):	0,0	0,2	0,4	0,8	1,4	2,0

b) Não é necessário repetir a curva a cada batelada (quando em trabalho continuado com as mesmas sol e condições de trabalho), mas somente a prova em branco e 2 padrões.

3.5.3.2 – Determinação de Nitrogênio

a) Pipetar 10 mL do extrato para balão de destilação de 100 mL.	a) Ajustar a vazão do vapor a 35 - 40 mL em 3 a 4 min.
b) Adicionar 5 mL de NaOH 10M e iniciar a destilação imediatamente.	b) Receber o destilado em erl de 50 mL contendo 5 mL de indicador-ac bórico.
c) Destilar até coletar 35-40 mL.	
d) Titular com H_2SO_4 0,025M.	d) Usar microbureta de 5 mL.

3.5.3.2.1 – Observações

a) Iniciar com a prova em branco e observar se o valor obtido é aceitável.

b) A determinação de N é geralmente feita por último, não sendo afetada pela presença do precipitado na alíquota.

c) O tempo requerido para a digestão, em geral, é menor nas análises de tecido, devido à menor quantidade de compostos refratários presentes.

d) Cada mL de H_2SO_4 0,025M utilizado na titulação (descontado o branco) corresponde a 700 µg de N. Se a concentração do ac utilizado for diferente desta, calcular por proporção direta o fator a utilizar nos cálculos.

e) A sensibilidade do método (com as especificações dadas) é de 0,017% de N (para 0,01 mL de ac utilizado na titulação). Maior sensibilidade pode ser obtida destilando 20 mL do extrato.

f) Para maiores detalhes quanto à operação do destilador semi-micro-Kjeldahl consultar o capítulo 2.14.

3.5.3.2.2 – Cálculos

a) Utilizar a fórmula:

$$\% N = \frac{(mL\ H^+_{am} - mL\ H^+_{br}) \times 700 \times 5 \times 5}{10.000}$$

(no caso de utilizar 0,200 g da amostra, destilando-se 10 mL do extrato (após a diluição a 50 mL) e titulando-se com H_2SO_4 0,025M).

b) Expressar o resultado em % de N ($m\ m^{-1}$), com 2 dígitos decimais.

3.5.3.3 – Determinação de fósforo

a) Transferir uma alíquota de 1 mL do extrato para copo plástico descartável.	a) Usar ser calib.
b) Adicionar 2 mL de água dest.	b) Usar ser calib.
c) Adicionar 3 mL de sol P-B.	c) Usar ser calib.
d) Adicionar 3 gotas de sol P-C.	
e) Agitar e determinar a abs em 660 nm após 15 min.	e) Após 20-35 min em dias frios.

3.5.3.3.1 – Observações

a) Utilizar as mesmas ser para a preparação das amostras e padrões.

b) Amostras com alto teor de P (abs situada fora da parte reta da curva padrão) devem ser diluidas convenientemente com o extrato da prova em branco.

c) A sensibilidade do método (abs = 0,002) corresponde a ≈ 0,0015% de P na amostra. Pode-se determinar até ≈ 0,66% de P na amostra sem diluição.

d) Para leitura direta, pode-se ajustar o valor de 0,30% com o padrão de 2,0 mg L^{-1} de P.

3.5.3.3.2 – Cálculos

a) Curva padrão de P:

cf item 2.4.5.a1.

b) Fator de concentração: determinado pela curva padrão

c) Fator de diluição:

$$fd = \frac{50}{0,20} \times \frac{3}{1} \times \frac{6}{3} = 1.500$$

(a diluição pelo P-C não sendo considerada)

d) Teor de P:

$$P\ (\%) = \frac{\text{leitura} \times fc \times fd}{10.000}$$

e) Expressar o resultado em % de P (m m^{-1}), com 2 dígitos decimais.

3.5.3.4 – Determinação de potássio

a) Retirar uma alíquota de 1 mL e transferir para um copo plástico descartável.	a) Usar ser calib.
b) Adicionar 10 mL de H_2O dest.	
c) Determinar a emissão de luz no fotômetro de chama.	

3.5.3.4.1 – Observações

a) Nos fotômetros que dispõem de controle de sensibilidade, ajusta-se o máximo da escala com padrão adequado conforme as amostras. Se houver amostras com baixo teor, estas podem ser determinadas utilizando como ajuste máximo um padrão intermediário. As amostras com teor alto podem ser analisadas utilizando como ajuste máximo o padrão maior. Evita-se assim a necessidade de diluir as amostras.

b) A sensibilidade do método (leitura = 1), para o caso de ajustar o ponto 100 da escala com o padrão de 8 mg L^{-1} de K na solução final corresponde a 0,022% de K na amostra. O teor máximo que pode ser determinado com a diluição recomendada é de 5,5% de K na amostra (ajustando-se a leitura 100 com o padrão de 20 mg L^{-1}).

c) Para leitura direta em fotômetros com escala iluminada fixa pode-se desenhar uma escala (em %) sobreposta à escala do aparelho, ajustando-se o valor de 2,2% para o padrão de 8,0 mg L^{-1}.

3.5.3.4.2 – Cálculos

a) Curva padrão de K:

cf item 2.4.5b.

b) Concentração na sol final (cs), determinada pela curva padrão.

c) Fator de diluição:

$$fd = \frac{50}{0,20} \times \frac{11}{1} = 2.750$$

d) Teor de K:

$$K\ (\%) = \frac{\text{leitura} \times cs \times fd}{10.000}$$

e) Expressar o resultado com 2 dígitos decimais (em % m m^{-1}).

3.5.3.5 – Determinações de cálcio e magnésio

a) Transferir uma alíquota de 2,5 mL do extrato para copo plástico descartável.	a) Usar ser calib.
b) Adicionar 2,5 mL de H_2O dest.	
c) Adicionar 5 mL da sol de Sr 0,3% em HCl 0,2M.	
d) Determinar a abs do Ca no fotômetro de absorção.	d) Observar as instruções peculiares a cada aparelho.
e) Retirar uma alíquota de 5 mL.	e) Do copo plástico após a leitura da abs do Ca.
f) Adicionar 10 mL de água dest.	
g) Determinar a abs do Mg no fotômetro de absorção.	

3.5.3.5.1 – Observações

a) Amostras com altos teores de Ca e Mg devem ser diluídas convenientemente com o extrato da prova em branco (2 mL do extrato, 2 mL de H_2O dest e 4 mL da sol de Sr).

b) Com a metodologia adotada é possível determinar entre 0,002% e 0,83% de Ca e entre 0,001 e 0,57% de Mg na amostra, sem necessidade de diluição.

c) Para a leitura direta do teor de Ca, ajustar o aparelho para 0,80% de Ca com o padrão de 8,0 mg L^{-1}.

d) Para a leitura direta do teor de Mg, ajustar o aparelho para 0,24% de Mg como o padrão de 0,8 mg L^{-1}.

3.5.3.5.2 – Cálculos

a) Cálcio

a.1) curva padrão de Ca:

cf item 2.7.3.3.2a.1.

a.2) fator de concentração: determinado pela curva padrão

a.3) fator de diluição:

$$fd = \frac{50}{0,20} \times \frac{5}{2,5} \times \frac{10}{5} = 1.000$$

a.4) teor de Ca:

$$Ca\ (\%) = \frac{\text{leitura} \times fc \times fd}{10.000}$$

a.5) expressar o resultado com 2 dígitos decimais (% m m^{-1}).

b) Magnésio

b.1) curva padrão de Mg:

cf item 2.7.3.3.2b.1.

b.2) fator de concentração: determinado pela curva padrão

b.3) fator de diluição:

$$fd = \frac{50}{0.20} \times \frac{5}{2.5} \times \frac{10}{5} \times \frac{15}{5} = 3.000$$

b.4) teor de Mg:

$$Mg\ (\%) = \frac{\text{leitura} \times fc \times fd}{10.000}$$

b.5) expressar o resultado com 2 dígitos decimais (% m m^{-1}).

II Laboratory protocols for extracting N total and mineral of the soil

2.14 – NITROGÊNIO TOTAL NO SOLO

O nitrogênio é encontrado na natureza como gás (N_2) muito pouco reativo (energia de dissociação de 225,8 Kcal mol^{-1}) ou combinado com outros elementos, principalmente oxigênio, hidrogênio e carbono em ligações covalentes. Os íons NH_4^+, NO_3^-, NO_2^- podem ser facilmente determinados por destilação ou colorimetria. Os gases nitrogenados mais reativos como NO e NO_2 são também de fácil determinação, o que não ocorre entretanto com N_2O e N_2.

Os métodos de avaliação do N total mais utilizados são o método de Kjeldahl (oxidação úmida) e o método de Dumas (oxidação seca ou combustão).

Devido à facilidade de execução, utilização de equipamento simples e baixo custo das análises, o método de Kjeldahl é usado preferencialmente. Estão disponíveis também aparelhos semi-automatizados de combustão seca que apresentam entretanto as desvantagens de alto custo e dependência de reativos da fábrica.

O procedimento comentado a seguir é baseado no método descrito por J. KJELDAHL em 1883, com pequenas modificações para aumentar a sensibilidade e exatidão do método, além do uso de equipamento mais adequado. Uma discussão detalhada de cada fase desse procedimento é dada por BREMNER (1965) e BREMNER & MULVANEY (1982).

No método de Kjeldahl, o N da amostra é reduzido a NH_4^+ pela digestão com H_2SO_4 concentrado. Para as proteínas por exemplo, a reação é:

$$-\underset{|}{\overset{|}{C}}-NH_2 + H_2SO_4 \longrightarrow NH_4^+ + SO_4^{2-} + CO_2\uparrow + SO_2\uparrow$$

Para aumentar a rapidez e a eficiência da conversão do N orgânico em $N\text{-}NH_4^+$ pela digestão com H_2SO_4 adicionam-se: a) sais, como K_2SO_4 ou Na_2SO_4, para aumentar a temperatura da digestão e, b) catalizadores para facilitar a oxidação da matéria orgânica, como Se, Hg ou Cu.

Neste método, a digestão com H_2SO_4 não é completa para o N orgânico de compostos heterocíclicos refratários (p.ex. piridinas), e de certos compostos contendo ligações N-N e N-O (p.ex. N_2H_4, NO_3^-). Com exceção de NO_3^- todavia, estes compostos não são encontrados em quantidades suficientes para alterar os resultados, e os procedimentos a seguir descritos fornecem resultados suficientemente exatos para as análises de N total no solo e em plantas. Em solos, o teor de NO_3^- é geralmente insignificante em relação ao N total. O tecido vegetal todavia em alguns casos pode conter alta percentagem de NO_3^-. Deve-se, neste caso, usar o método modificado, para inclusão do NO_3^- (descrito no item 2.14.3.3).

Devido à maior eficiência, foi adotado o método de digestão em tubos de ensaio (25 x 250 mm) com bloco digestor (350-375°C), mantendo-se a mistura de digestão (com Na_2SO_4) e catalizador nas proporções recomendadas por BREMNER & MULVANEY (1982). Para as amostras com alto teor de nitrato (acima de 10% do valor de N total) é recomendado o método modificado com redução prévia de nitrato por permanganato e Fe reduzido (BREMNER & SHAW, 1958).

Para a destilação do N deve-se alcalinizar a solução:

$$NH_4^+ + OH^- \rightleftharpoons \uparrow NH_3 + H_2O$$

Na destilação, o vapor de água favorece (por arraste e agitação) a volatilização da amônia. No condensador a reação anterior procede da direita para a esquerda, recolhendo-se então o condensado em solução de ácido bórico-indicador:

$$H_3BO_3 + OH^- \rightleftharpoons H_2BO_3^- + H_2O$$

A titulação é feita com H_2SO_4 diluído:

$$H^+ + H_2BO_3^- \rightleftharpoons H_3BO_3$$

O indicador misto de verde de bromocresol e vermelho de metila em ac bórico possibilita a fácil observação da mudança de cor na titulação, que ocorre em intervalo menor do que uma unidade de pH (de 4,8 a 5,6).

A adaptação de uma agulha hipodérmica fina (cortada em ângulo reto para obter gotas pequenas, de 0,01 mL) à microbureta de 5 mL usada na titulação com H_2SO_4 0,025M possibilita uma sensibilidade de 7 µg de N (erro de 0,12% numa amostra de 0,200 g de um material com 2% de N).

Devido à pequena quantidade de solo utilizada na análise, o erro de amostragem deve ser controlado por: a) moagem da amostra até passar em peneira de 50 mpp (0,30 mm); b) homogeneização da amostra moída.

2.14.1 – Material

a) Bloco digestor com 40 provas para tubos de ensaio de 25 x 250 mm em Pyrex, com controle eletrônico de temperatura (até 400°C).

b) Manta elétrica para produção de vapor, com frasco de fundo redondo de 5 litros. É aconselhado o uso de um reostato para controle da produção de vapor. Pode ser também utilizado um balão de pirex de 2 L, com resistência elétrica no interior e controle eletrônico de intensidade de calor.

c) Agitador magnético para a titulação.

d) Destilador a vapor semi-micro-Kjeldahl, proposto por BREMNER & EDWARDS (1965), modificado (Fig. 1) por TEDESCO & GIANELLO (1979) com frascos de digestão-destilação de 100 mL (com junção de vidro esmerilado 19/38) - (TECNOGLASS - P. Alegre).

e) Microbureta de 5 mL com carregamento automático (graduação de 0,01 mL).

2.14.2 – Soluções

a) H_2SO_4 concentrado (d = 1,84).

b) NaOH 10M: pesar 400 g de NaOH e dissolver em 800 mL de água dest em copo de becker. Após esfriar, transferir para um balão vol e completar o volume a 1.000 mL.

c) Mistura de digestão: moer em almofariz separadamente 100 g de Na_2SO_4, 10 g de $CuSO_4.5H_2O$ e 1 g de selênio (metálico). Misturar bem, e moer novamente a mistura (o Se em geral já é adquirido em pó).

d) Solução de indicador-ac bórico: dissolver 40 g de ac bórico (o produto comercial pode ser usado se não for feita a determinação isotópica de ^{15}N) em ≈ 1.400 mL de água quente. Após esfriar, transferir para um balão vol de 2 L contendo 400 mL de etanol 95% (produto técnico) e 40 mL de uma solução obtida pela dissolução de 0,660 g de verde de bromocresol e 0,330 g de vermelho de metila em 1.000 mL de etanol 95%. Misturar as soluções no balão volumétrico e adicionar cuidadosamente NaOH 0,05M até que mal se observe uma leve mudança de cor de roxo para verde claro ao adicionar 1 mL de água dest a 1 mL do indicador. Completar a seguir o volume a 2 L com água dest e misturar. Pode-se também determinar o pH da solução, que deve ficar entre 5,0 e 5,1.

e) H_2SO_4 0,025M: dissolver 1,4 mL de H_2SO_4 conc a 1 L com água dest (1 mL desta solução gasto na titulação corresponde a 700 μg de N [ver item 2.14.3.4 para a padronização]).

f) Solução de $KMnO_4$ 5%: dissolver 50 g de $KMnO_4$ em 1.000 mL de água dest e guardar em frasco escuro.

g) H_2SO_4 50%: adicionar (vagarosamente e agitando) 500 mL de H_2SO_4 conc a 500 mL de água dest.

h) Fe reduzido (moído até passar em peneira de 100 mpp).

2.14.3.1 – Operação dos destiladores

A operação correta dos destiladores requer alguns cuidados, detalhados a seguir, para a melhor eficiência de trabalho e evitar contaminação nas análises.

a) Usar ≈ 4,3 L de água dest no balão de 5 L (de fundo redondo) para produção de vapor, ao qual se acrescenta 1 mL de H_2SO_4 conc (para evitar a volatização de NH_3 presente na água como impureza). À medida que baixa o nível da água no balão pela formação de vapor, completar o volume. Trocar a água a cada 15 dias aproximadamente, quando em uso intenso. No caso de ser utilizado balão com resistência elétrica, não adicionar ácido. Dependendo do teor de NH_3 da água poderá neste caso ocorrer contaminação de N.

b) Colocar no balão de vapor algumas esferas de vidro para favorecer a ebulição (pode-se também usar fragmentos de "pedra pomes" com esta finalidade).

c) A taxa de produção de vapor deve ser ajustada para a coleta de 35-40 mL de água no condensador em 4 min.

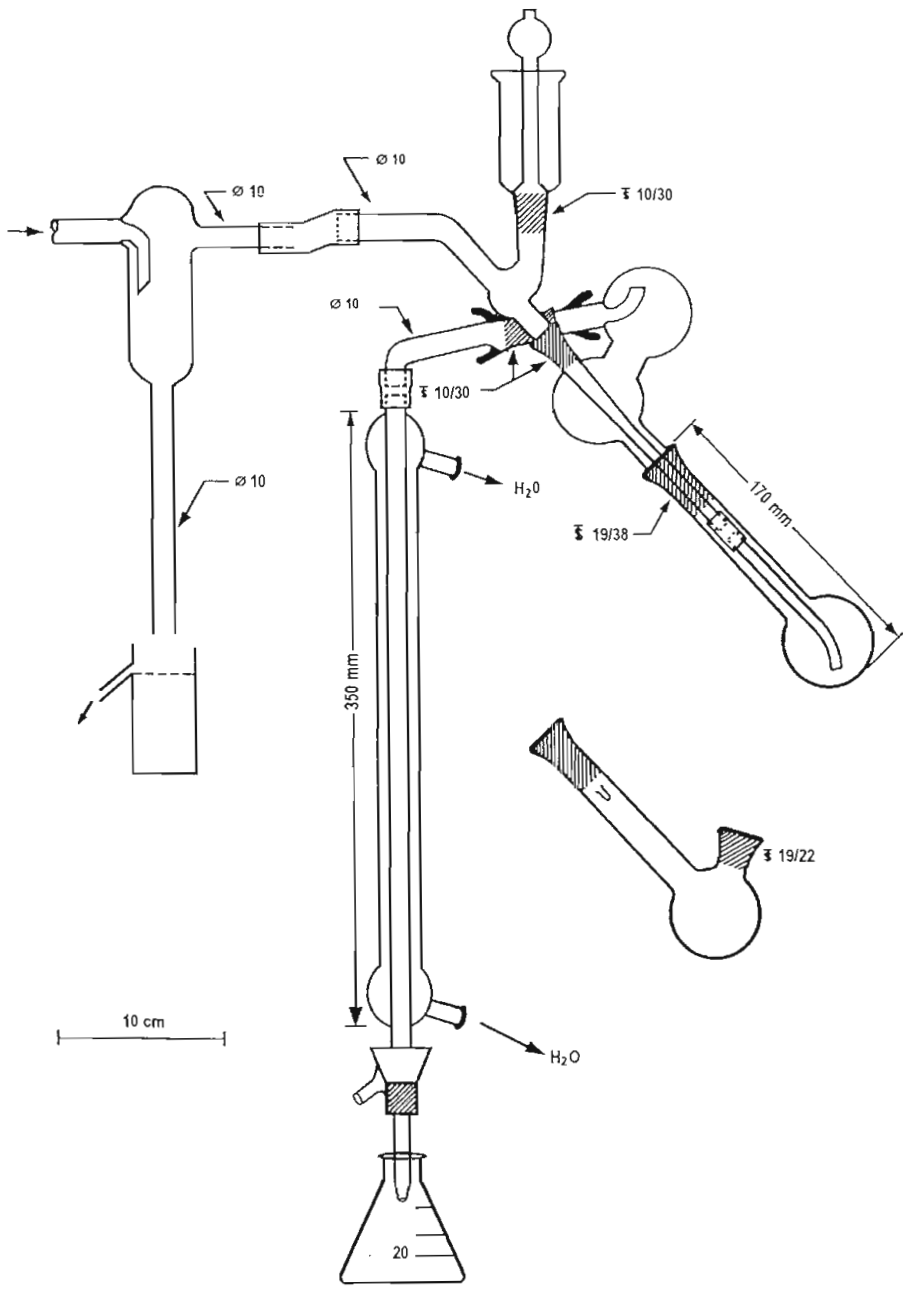

Figura 1 – Conjunto modificado de destilação de amônia por arraste de vapor (TEDESCO & GIANELLO, 1979), com coluna d'água para controlar a saída de vapor.

d) O fluxo de água para resfriamento do condensador deve ser ajustado para que a temperatura do condensado seja inferior a 22°C, caso contrário poderão ocorrer perdas de NH_3.

e) Antes de iniciar as análises, operar o destilador por 10-15 min a fim de assegurar que o mesmo esteja limpo.

f) Para testar a limpeza do aparelho, destilam-se 20 mL de água dest; o indicador ac bórico deve tornar-se cor verde claro com os primeiros mililitros de destilado. O valor da titulação deste destilado deve estar compreendido na variação usual obtida neste teste (que varia com o ponto de titulação do indicador e a concentração do ácido), e próximo à prova em branco.

g) Sempre destilar e titular a prova em branco (contendo somente os reativos e/ou soluções) antes das amostras. Se for obtido um valor muito diferente do usual, eliminar a fonte de contaminação antes de prosseguir o trabalho.

h) O destilador sempre apresenta um pequeno efeito de "memória" nas análises devido à adsorção de NH_3 nas paredes da vidraria. Isto pode provocar um erro sério somente quando: a) for feita uma destilação de amostra com baixo teor de N após uma amostra com alto teor de N; e, b) há necessidade da determinação isotópica de ^{15}N. Nestes casos deve-se lavar o aparelho após cada análise destilando-se 10-15 mL de álcool 95%.

i) Se ocorrer o arraste do material no frasco de destilação para dentro do destilador, este pode ser lavado com H_2SO_4 diluído (destilando cuidadosamente 45 mL de ácido num frasco de 50 mL). O aparelho deve ser a seguir lavado da mesma forma diversas vezes com água dest. Operar depois o destilador (sem amostra) por no mínimo 15 min. Testar então a limpeza do aparelho como foi descrito no item f.

j) A limpeza dos frascos de destilação de nitratos (em que se utiliza a liga Devarda) pode ser feita eficientemente com solução de limpeza (após uma lavagem prévia com água). Os frascos utilizados na determinação de N total podem ser limpos eficientemente com uma solução de ácido 2M (após uma lavagem prévia com água).

k) A quantidade de ácido gasto na titulação da prova em branco (somente os reativos) deve ser subtraída do valor da titulação das amostras.

2.14.3.2 – Determinação do Nitrogênio Total

(para amostras com baixo teor de nitrato)

a) Pesar 0,500 g de solo mineral e colocar em tubo de ensaio de 25 x 250 mm.	a) Para solos orgânicos (MO > 8% utilizar 0,200 g).
b) Adicionar 1 mL de H_2O_2 30%.	b) Usar ser.
c) Adicionar 2,0 mL de H_2SO_4.	c) Usar ser.
d) Deixar esfriar 15-10 min.	d) Ou 5 min em água fria.

e) Adicionar 0,7 g de mistura de digestão.	e) Usar medida calib e funil de haste longa.
f) Colocar no bloco digestor e elevar a temperatura a 250°C. Manter a esta temperatura por 15 a 20 min.	f) Este aquecimento deve ser gradual; começar com o bloco frio ou abaixo de 150°C.
g) Elevar a temperatura a 350-375°C.	g) Marcar o tempo em que as amostras perdem a coloração preta, ficando cor de palha.
h) Após clarear (cor amarelo-esverdeado), manter a 350-375°C por 2 horas.	h) Ver obs 2.14.3.2.1b.
i) Deixar esfriar sobre uma placa de amianto ou madeira.	i) Até poder tocar o fundo dos tubos (50-60°C).
j) Adicionar 5 mL de água dest e agitar.	j) Se esfriar demais, o extrato solidifica (deve-se neste caso aquecer em banho-maria, não no bloco digestor).
k) Transferir quantitativamente para um frasco de destilação de 100 mL, utilizando 20 a 30 mL de água dest.	k) Cuidar para não perder extrato na transferência.
l) Conectar o frasco ao destilador, e com a coluna de água abaixada, adicionar vagarosamente pelo funil 10 mL de NaOH 10M.	l) O destilador já deve estar limpo (ver obs 2.14.3.2.1a). O NaOH é colocado no funil com pipetador de Kipp.
m) Levantar a coluna de água e destilar em 5 mL de indicador-ac bórico.	m) O indicador pode ser medido com pipetador de Kipp ou ser.
n) Após coletar 35-40 mL de destilado, parar a destilação e titular com H_2SO_4 0,025M.	n) A cor muda de verde a rosa. Usar microbureta de 5 mL e agitador magnético.

2.14.3.2.1 - Observações

a) Antes das amostras, destilar e titular a prova em branco.

b) Se a temperatura se elevar acima desse máximo (por aquecimento desuniforme do bloco digestor ou por defeito no controlador eletrônico), as amostras podem se solidificar. Se isso ocorrer, deixar os tubos esfriarem, adicionar 5-10 mL de H_2O dest e aquecer em banho-maria para ressolubilizar os sais. Se a solidificação ocorrer com muita freqüência, diminuir a temperatura do bloco digestor até que não ocorra mais a solidificação (não diminuir abaixo de 330°C).

c) Se o valor da titulação da prova em branco for muito diferente da variação normalmente encontrada, localizar o erro antes de iniciar a titulação das amostras.

d) Se o H_2SO_4 utilizado na titulação for exatamente 0,025M, cada mL gasto na titulação (subtraído o valor da prova em branco) corresponde a 700 μg de N. Se a normalidade do ácido for diferente, calcular o valor correspondente por proporção direta.

c) A sensibilidade do método (0,01 mL de ácido gasto e amostra de 0,5 g) é de 0,001%.

2.14.3.2.2 - Cálculos

a) Utilizar a fórmula:

$$\% N = \frac{mL\ H^{+}_{am} - mL\ H^{+}_{br}}{g\ solo \times 10.000} \times 700 \qquad \text{(para o ácido 0,025M)}.$$

b) Expressar o valor em % de N total com 3 dígitos decimais (% m m^{-1}).

2.14.3.3 - Determinação do Nitrogênio Total

(para amostras com alto teor de nitrato)

a) Pesar 0,500 g de solo mineral e colocar em tubo de ensaio de 25 x 250 mm.	a) Para solos orgânicos (MO > 8% utilizar 0,200 g).
b) Adicionar na seqüência: 1 mL de H_2O_2 30%; 1 mL de $KMnO_4$ 50%; 2 mL de H_2SO_4 50%.	b) Usar ser.
c) Depois de 5 min, adicionar 0,5 g de Fe reduzido.	c) Usar medida calib e funil de haste longa.
d) Digerir por 1 hora em temperatura inferior a 100°C.	d) Pode-se usar o bloco digestor ou banho-maria.
e) Adicionar 0,7 g de mistura de digestão.	e) Usar medida calib e funil de haste longa.
f) Adicionar 2 mL de H_2SO_4 conc.	f) Usar ser.
g) Colocar no bloco digestor e elevar a temperatura a 250°C. Manter a esta temperatura por 15-20 min.	
h) Elevar a temperatura a 350-375°C.	
i) Após clarear (cor amarelo-esverdeado) manter a 350-375°C por 2 horas.	i) Ver observação 2.14.3.2.1b.
j) Deixar esfriar sobre uma placa de amianto ou madeira.	j) Até poder tocar o fundo (50-60°C).
k) Adicionar 5 mL de água dest e agitar.	k) Evitar que o extrato solidifique.
l) Transferir quantitativamente para um frasco de destilação de 100 mL, utilizando 20 a 30 mL de água dest.	
m) Conectar o frasco ao destilador, e com a coluna d'água abaixada adicionar vagarosamente pelo funil 15 mL de NaOH 10M.	

n) Levantar a coluna d'água e destilar em 5 mL de indicador-ac bórico.	n) Medir o indicador com pipetador de Kipp ou ser.
o) Após coletar 35-40 mL de destilado, parar a destilação e titular com H_2SO_4 0,025M.	o) A cor muda de verde a rosa. Usar microbureta de 5 mL e agitador magnético.

2.14.3.3.1 – Observações

Cf o item 2.14.3.2.1.

2.14.3.3.2 – Cálculos

Cf o item 2.14.3.2.2.

2.14.3.4 – Padronização do ácido com TRIS

O TRIS ou THAM (Tris-(hidroximetil) amino metano) é um padrão primário para a estandartização de soluções diluídas de ácidos. Possui alto peso molecular, é incolor, não higroscópio e pode ser obtido com alta pureza. Não absorve CO_2 quer na forma sólida quer em solução. Seu pH no ponto de equivalência é de 4,7. As soluções são estáveis à temperatura ambiente por no mínimo 3 meses.

Para a padronização do ácido procede-se da seguinte maneira:

a) Pesar 0,7 g de TRIS (reagente Merck nº 8382) e secar por 1 hora a 102°C (não deixar nesta temperatura por mais de 2 horas).

b) Dissolver 0,6057 g do reagente seco em balão vol de 100 mL com água dest livre de CO_2.

c) Ajustar o volume e homogeneizar. A solução assim preparada é 0,050 $mol_c\ L^{-1}$.

d) Pipetar 10,00 mL da solução 0,050 $mol_c\ L^{-1}$ de TRIS para um frasco de erl de 125 mL (em duplicata).

e) Adicionar 10 mL de água dest (livre de CO_2).

f) Adicionar 5 mL do indicador-ac bórico e titular com o ácido. No ponto de viragem o indicador passa da cor verde-claro a rosa-claro permanente.

$$H^+\ (mol_c\ L^{-1}) = \frac{10{,}00 \times 0{,}050}{mL\ H^+}$$

Observação: para a titulação do ácido 0,0025M utilizar somente 2,00 mL da solução de TRIS 0,050 $mol_c\ L^{-1}$.

2.15 – NITROGÊNIO MINERAL NO SOLO

Na maioria dos solos, o N inorgânico se encontra na forma de NH_4^+ e NO_3^-. Geralmente o NO_2^- ocorre em quantidades muito pequenas (em solos alcalinos pode-se encontrar NO_2^- em níveis tóxicos para as plantas após uma aplicação de adubos amoniacais).

Alguns solos podem ter NH_4^+ "fixado" (não trocável) nos minerais de argila do tipo 2:1, o qual não é extraído por solução de KCl (que extrai o NH_4^+ trocável e da solução). O método de determinação de NH_4^+ fixado é descrito por SILVA & BREMNER (1966).

A avaliação de NH_4^+ trocável, NO_2^- e NO_3^- no solo é dificultada porque essas formas de N se modificam rapidamente pela mineralização da matéria orgânica (amonificação), nitrificação, desnitrificação, etc. As análises portanto deveriam ser feitas imediatamente após a coleta. Em condições de campo isto nem sempre é possível. O congelamento possibilita guardar as amostras por tempo indeterminado. A secagem não paralisa totalmente as reações microbianas (principalmente a mineralização).

A solução extratora, além de remover quantitativamente NH_4^+ trocável, NO_2^- e NO_3^-, deve impedir qualquer modificação de natureza química ou biológica dessas formas durante o período de extração, possibilitando guardar o extrato por um tempo suficiente para as análises. Tanto o NO_2^- como o NO_3^- são solúveis em água, mas o NH_4^+ trocável deve ser extraído com soluções salinas, utilizando-se geralmente KCl, que não interfere no método de determinação apresentado.

Os métodos de destilação a vapor descritos a seguir são simples, rápidos e precisos, tendo sensibilidade adequada para a maioria dos trabalhos em solos. São também livres de interferências de outros compostos orgânicos nitrogenados (proteínas, aminas, etc). Não há necessidade de filtrar os extratos para a destilação.

A liberação de NH_3 na destilação é obtida pela alcalinização do extrato com MgO:

$$MgO + H_2O \longrightarrow Mg^{2+} + 2OH^-$$

$$NH_4^- + OH^- \longrightarrow \uparrow NH_3 + H_2O$$

Após a destilação da NH_3 em presença de NO_2^- e NO_3^- (que não são destilados), adiciona-se a liga Devarda (50% Cu, 45% Al e 5% Zn) para reduzir esses a NH_4^+ o qual é a seguir destilado na forma de NH_3.

O NO_3^- é determinado por titulação diferencial, com tratamento prévio do extrato com ac sulfâmico, que destrói quantitativamente o NO_2^-:

$$HNO_2 + NH_2SO_3H \longrightarrow N_2 + H_2SO_4 + H_2O$$

Os métodos apresentados a seguir são os descritos por BREMNER & KEENEY (1966), com extração e destilação de uma alíquota do extrato, tendo-se as seguintes opções:

1) determinação conjunta de $NH_4^+ + NO_3^- + NO_2^-$;

2) determinação de NH_4^+ e $NO_3^- + NO_2^-$; e

3) determinação separada de cada um destes íons.

A extração do N mineral no solo é feita com KCl. BREMNER & KEENEY (1966) recomendam o uso de KCl 2M. Em nossas condições entretanto (solos com baixo teor de MO, menor CTC) o KCl 1M é eficiente na extração quantitativa do N mineral no solo.

2.15.1 – Material

A vidraria e aparelhos auxiliares (mantas, agitadores, etc) necessários são os mesmos descritos em 2.14.1. Na determinação concomitante de NH_4^+ e NO_3^- é conveniente utilizar os frascos de destilação com braço de carga lateral de 100 mL. É recomendado ter à mão também frascos de 200 mL, para algumas situações especiais (necessidade de usar uma alíquota de até 100 mL na destilação, extratos de solos que apresentam grande formação de espuma, etc).

2.15.2 – Soluções e reagentes

a) MgO: aquecer o MgO leve (produto técnico pode ser usado se não houver necessidade da determinação isotópica de ^{15}N) no forno a 700°C por 2 horas. Deixar esfriar e guardar em frasco bem tampado (para evitar a absorção de CO_2 do ar, com formação de carbonato, que interfere nas análises). Se o MgO foi calcinado há bastante tempo (mais de 6 meses), é aconselhável calciná-lo novamente antes de usar.

b) Liga Devarda: deve ser bem fina (passar em peneira de 100 mpp e ao menos 75% em peneira de 300 mpp), caso contrário triturar em moinho de bolas.

c) Ácido sulfâmico: dissolver 2 g do reagente puro em 100 mL de água dest. Guardar em refrigerador. Se não houver disponibilidade de reagente de boa qualidade, pode-se purificar o NH_2SO_3H por cristalização em água quente.

d) H_2SO_4 0,0025M: diluir 10x a solução (H_2SO_4 0,025M) preparada cf o item 2.14.2e; padronizar pelo procedimento descrito em 2.14.3.4. Um mL gasto na titulação equivale a 70 µg de N (se a concentração for exatamente 0,0025M).

e) KCl 1M: dissolver 74,5 g de KCl a 1 L com água dest.

f) Indicador-ac bórico:* dissolver 200 g de ac bórico em 8 L de água dest quente (50-60°C). Esfriar, ajustar o volume e transferir para um frasco de volume adequado. Adicionar 1 L de etanol 95% e a solução de indicador preparada dissolvendo 0,132 g de verde de bromocresol e 0,066 g de vermelho de metila em 1 L de etanol 95%. Agitar e titular adicionando cuidadosamente NaOH 0,05M até que mal se observe uma leve mudança de cor de roxo para verde claro ao adicionar 1 mL de água dest a 1 mL do indicador. Esta solução pode ser conservada indefinidamente (podem ser utilizados o ac bórico técnico e o etanol 95% comercial, caso não haja necessidade da determinação isotópica de ^{15}N).

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*uma alternativa do procedimento descrito anteriormente (2.14.2d), para preparar 10 L de indicador, suficiente para 2.000 análises.

2.15.3.1 – Extração de NH_4^+, NO_3^- e NO_2^- do solo

a) Pesar 5 g de solo em frasco "snap-cap" de 90 mL.	a) Medir o solo, se necessário expressar o resultado em teor por volume.
b) Adicionar 50 mL de KCl 1M.	
c) Agitar 30 min.	
d) Deixar decantar por 30 min.	
e) Pipetar alíquotas adequadas do extrato.	e) conforme os procedimentos descritos nos ítens 2.15.3.2 e subseqüentes.

2.15.3.1.1 – Observações

a) Se a destilação não for feita dentro de 24 horas, filtrar o extrato e guardar o filtrado em refrigerador (≈ 4°C) para posterior destilação.

b) Se houver necessidade de usar menor relação solo:solução deve-se aumentar a concentração do KCl (p. ex. 5 mL de KCl 2M por g de solo).

c) O método mais indicado para a avaliação do N mineral a campo (em experimento) é a extração no próprio local. Pesam-se 30-40 g da amostra recém colhida e bem homogeneizada (com balança de sensibilidade de 0,1 g) e coloca-se a sub-amostra em garrafas ou frascos de boca larga contendo 200 mL de KCl 1M (pesa-se também outra sub-amostra para determinação da umidade). Se as análises forem feitas no período de 24 horas, utiliza-se simplesmente uma alíquota do extrato; caso contrário, deve-se filtrar e guardar o filtrado em refrigerador (≈ 4°C).

d) É possível também determinar o N mineral no solo por destilação direta (KEENEY & BREMNER, 1966) colocando-se 2 g de solo no frasco de destilação contendo 20 mL de KCl 1M, e destilando-se a seguir. Em solos com alto teor de MO, entretanto, este procedimento é dificultado pela excessiva formação de espuma no extrato após a adição de liga Devarda.

2.15.3.2 – Determinação do N mineral ($NH_4^+ + NO_3^- + NO_2^-$)

a) Pipetar uma alíquota de 20 mL do extrato para um frasco de destilação de 100 mL.	a) Alíquota não deve conter mais de 2 mg de N.
b) Ajustar no condensador um frasco de erl de 50 mL contendo 5 mL de indicador-ac bórico.	b) Não há necessidade de mergulhar a ponta do condensador na solução do indicador.
c) Adicionar 0,2 g de MgO e 0,2 g de liga Devarda.	c) Usar um funil de haste longa com Ø de 1 cm e medidas calib.

d) Conectar imediatamente o frasco no destilador e iniciar a destilação levantando a coluna d'água.	
e) Recolher 35-40 mL de destilado no frasco de erl.	e) O destilado contém a soma de NH_4^+, NO_3^- e NO_2^- presentes na alíquota destilada.
f) Parar a destilação e titular o destilado.	f) A coloração do indicador que se torna verde-clara com as primeiras gotas do destilado passa para rosa-claro no ponto de viragem da titulação.

2.15.3.2.1 – Observações

a) Antes das amostras, destilar e titular a prova em branco.

b) Se o valor da titulação da prova em branco for muito diferente da variação normalmente encontrada, localizar o erro antes de iniciar a titulação das amostras.

c) A sensibilidade do método descrito é de 0,035 mg L^{-1} na solução ou 0,35 mg kg^{-1} no solo (incerteza na titulação de 2 gotas H_2SO_4 0,0025M, microbureta de leitura de 0,01 mL com gotas de 0,01 mL, relação solo:solução de 1:10 e alíquota destilada de 20 mL). Uma sensibilidade maior pode ser obtida pela redução da relação solo:solução (aumentando porém a concentração do extrator) ou destilando-se uma alíquota maior do extrato (até 100 mL, utilizando o frasco de destilação de 200 mL).

d) Se a concentração do H_2SO_4 for diferente de 0,0025M, calcular por proporção direta o número de μg de N equivalentes a 1 mL gasto na titulação (descontado o branco).

2.15.3.2.2 – Cálculos

a) Utilizar a fórmula:

$$\text{N mineral (mg kg}^{-1}) = \frac{(\text{mL } H^+_{am} - \text{mL } H^+_{br}) \times 70 \times 2,5}{5 \text{ g}}$$

(para H_2SO_4 0,0025M, 5 g de solo extraído com 50 mL de KCl 1N e destilação de 20 mL do extrato).

b) Expressar o resultado em número inteiro (mg kg^{-1}).

2.15.3.3 – Determinação de NH_4^+ e $NO_3^- + NO_2^-$

a) Pipetar uma alíquota de 20 mL do extrato para um frasco de destilação de 100 mL.	a) Usar o frasco com braço de carga lateral. A alíquota não deve conter mais de 2 mg de N.
b) Ajustar no condensador um frasco de erl de 50 mL contendo 5 mL de indicador-ac bórico.	b) Não há necessidade de mergulhar a ponta do condensador na solução do indicador.
c) Adicionar 0,2 g de MgO.	c) Usar um funil de haste longa e Ø interno de 1 cm e medida calib.
d) Conectar imediatamente o frasco no destilador e iniciar a destilação levantando a coluna d'água.	
e) Recolher 35-40 mL de destilado no frasco de erl.	e) O destilado contém o NH_4^+ presente na alíquota destilada.
f) Parar a destilação baixando a coluna d'água.	
g) Ajustar no condensador outro frasco de erl de 50 mL contendo 5 mL de indicador-ac bórico.	
h) Adicionar 0,2 g de liga Devarda pelo braço de carga lateral do frasco de destilação.	h) Usar outro funil de haste curta e Ø interno de 1 cm e medida calibrada.
i) Fechar imediatamente o braço de carga lateral do frasco e iniciar a destilação.	
j) Recolher 35-40 mL do destilado no frasco de erl.	j) O destilado contém o $NO_3^-+NO_2^-$ presentes na alíquota destilada.
k) Parar a destilação e titular o destilado.	k) A coloração do indicador que se torna verde-clara com as primeiras gotas do destilado, passa para rosa-claro no ponto de viragem da titulação.

2.15.3.3.1 – Observações

Cf o item 2.15.3.2.1.

2.15.3.3.2 – Cálculos

Cf o item 2.15.3.2.2, especificando neste caso os teores de NH_4^+, $NO_3^- + NO_2^-$ (em mg kg^{-1}).

2.15.3.4 – Determinação de NH_4^+, NO_3^- e NO_2^-

a) Destilar uma alíquota de 20 mL do extrato e executar o procedimento descrito em 2.15.3.3.	a) Determina-se assim o NH_4^+ e a soma de NO_3^- e NO_2^-.
b) Pipetar outra alíquota de 20 mL do extrato para um frasco de destilação de 100 mL.	b) Usar o frasco com braço de carga lateral.
c) Adicionar 1 mL da solução de ac sulfâmico e agitar com cuidado por alguns minutos.	c) O NO_2^- é decomposto pelo ac sulfâmico.
d) Adicionar 0,2 g de MgO e destilar até recolher 35-40 mL de destilado.	d) Não há necessidade de titular este destilado.
e) Ajustar no condensador um frasco de erl de 50 mL contendo 5 mL de indicador-ac bórico.	e) Não há necessidade de mergulhar a ponta do condensador na solução do indicador.
f) Adicionar 0,2 g de liga Devarda pelo braço de carga lateral do frasco de destilação.	f) Usar outro funil de haste curta e Ø interno de 1 cm e medida calib.
g) Fechar imediatamente o braço de carga lateral e iniciar a destilação.	
h) Recolher 35-40 mL do destilado no frasco de erl.	h) O destilado contém o NO_3^- presente na alíquota destilada.
i) Parar a destilação e titular o destilado.	i) A coloração do indicador que se torna verde-clara com as primeiras gotas do destilado, passa para rosa-claro no ponto de viragem da titulação.

2.15.3.4.1 – Observações

Cf o item 2.15.3.2.1.

2.15.3.4.2 – Cálculos

Cf o item 2.15.3.2.2, obtendo-se o teor de NO_2^- por diferença entre as duas destilações com liga Devarda, e especificando neste caso, separadamente, os teores de NH_4^+, NO_3^- e NO_2^- (em mg kg^{-1}).

Appendix B

Additional graphics

Table B.1: Averages and standard deviations of biomass and nutrient levels of the various soybean organs and soil nitrogen levels studied at the beginning of flowering (R1 stage) under grazing and fertilisation treatments. Results of contrast test is exposed with "A" and "B" for pasture modalities and "$\triangle$" and "$\bigtriangledown$" for fertilisation modalities.

R1	**Variable**	**Grazed**		**Non-grazed**		**P-values**		
		Systemic	**Conventional**	**Systemic**	**Conventional**	**Pasture**	**Fertilisation**	**Interaction**
Plant	Nodule number	102 ±31.3	96.8 ±40.9	105 ±39.0	96.8 ±25.5	0.859	0.661	0.901
	Nodule biomass (g)	0.451 ±0.186	0.429 ±0.178	0.418 ±0.176	0.429 ±0.173	0.519	0.666	0.647
	Root biomass (g)	4.01 ±0.965	4.06 ±1.46	3.71 ±0.921	3.57 ±0.866	0.231	0.207	0.435
	Vegetative biomass (g)	33,7 ±12.9	29.3 ±8.71	29.4 ±8.22	30.3 ±12.0	0.344	0.338	0.435
	N% leaves + stem	2.23 ±0.502	2.10 ±0.490	2.20 ±0.515	2.49 ±0.420	0.935	0.726	0.404
	P% leaves + stem	0.326 ±0.021	0.311 ±0.023	0.325 ±0.037	0.330 ±0.032	0.933	0.274	0.312
	K% leaves + stem	1.83 ±0.343	2.19 ±0.514	2.11 ±0.298	2.19 ±0.212	0.112	0.041*	0.258
	N leaves + stem (g)	0.715 ±0.188	0.614 ±0.211	0.617 ±0.123	0.77 ±0.24	0.406	0.391	0.127
	P leaves + stem (g)	0.11 ±0.041	0.09 ±0.020	0.10 ±0.034	0.104 ±0.039	0.561	0.382	0.396
	K leaves + stem (g)	0.631 ±0.300	0.638 ±0.220	0.616 ±0.185	0.711 ±0.331	0.933	0.969	0.725
Soil	N Total soil (g/kg)	1.37 ±0.053	1.40 ±0.225	1.20 ±0.168	1.5 ±0.374	0.366	0.866	0.194
	NO3 soil (g/kg)	0.754 ±0.255	0.759 ±0.133	0.449 ±0.165	0.725 ±0.187	0.040*	0.974	0.197
	NH4 soil (g/kg)	$0.0564^{A} \pm 0.018$	0.0568 ±0.007	$0.0354^{B} \pm 0.012$	0.0524 ±0.012	0.035*	0.966	0.239

Table B.2: Averages and standard deviations of biomass and nutrient levels of the various soybean organs and soil nitrogen levels studied at the end of grain filling (R5.5 stage) under grazing and fertilisation treatments. Results of contrast test is exposed with "A" and "B" for pasture modalities and "△" and "▽" for fertilisation modalities.

R5.5	**Variable**	**Grazed**		**Non-grazed**		**P-values**		
		Systemic	**Conventional**	**Systemic**	**Conventional**	**Pasture**	**Fertilisation**	**Interaction**
Plant	Pod number	$86.5^{A} \pm 14.6$	86.0 ±21.3	$76.2^{B\bigtriangledown} \pm 21.3$	$86.4^{\bigtriangleup} \pm 23.9$	0.019 *	0.610	0.046 *
	Pod biomass (g)	$28.1^{A} \pm 4.53$	27.3 ±6.62	$23.8^{B} \pm 7.06$	26.9 ±6.25	0.026 *	0.696	0.269
	Vegetative biomass (g)	43.6 ±7.39	44.9 ±9.42	38.5 ±10.8	43.4 ±10.2	0.057	0.638	0.344
	N% pod	3.95 ±0.222	3.87 ±0.271	3.82 ±0.255	3.77 ±0.273	0.220	0.457	0.832
	P% pod	0.362 ±0.093	0.363 ±0.091	0.311 ±0.146	0.332 ±0.140	0.299	0.973	0.773
	K% pod	1.85 ±0.191	1.99 ±0.303	1.96 ±0.304	1.98 ±0.185	0.286	0.196	0.421
	N pod (g)	1.14 ±0.160	1.10 ±0.269	0.962 ±0.231	1.05 ±0.355	0.195	0.793	0.520
	P pod (g)	0.104 ±0.030	0.104 ±0.040	0.081 ±0.046	0.093 ±0.050	0.177	0.984	0.619
	K pod (g)	0.533 ±0.090	0.555 ±0.117	0.488 ±0.105	0.549 ±0.176	0.447	0.696	0.647
	N% leaves + stem	1.54 ±0.209	1.49 ±0.223	1.63 ±0.317	1.50 ±0.388	0.487	0.629	0.673
	P% leaves + stem	0.322 ±0.062	0.312 ±0.065	0.300 ±0.059	0.300 ±0.063	0.399	0.689	0.777
	K% leaves + stem	1.19 ±0.375	1.39 ±0.293	1.36 ±0.287	1.53 ±0.331	0.278	0.209	0.920
	N leaves + stem (g)	0.706 ±0.140	0.697 ±0.226	0.628 ±0.112	0.687 ±0.262	0.431	0.932	0.628
	P leaves + stem (g)	0.147 ±0.033	0.143 ±0.042	0.117 ±0.028	0.138 ±0.051	0.107	0.840	0.346
	K leaves + stem (g)	0.546 ±0.202	0.646 ±0.227	0.533 ±0.131	0.718 ±0.331	0.906	0.375	0.588
	N plant (g)	1.84 ±0.238	1.80 ±0.463	1.59 ±0.291	1.74 ±0.559	0.235	0.836	0.527
	P plant (g)	0.251 ±0.036	0.247 ±0.074	0.198 ±0.055	0.231 ±0.084	0.044 *	0.876	0.324
	K plant (g)	1.08 ±0.263	1.20 ±0.320	1.02 ±0.221	1.27 ±0.490	0.722	0.450	0.588
Soil	N Total soil (g/kg)	1.32 ±0.056	1.48 ±0.169	1.52 ±0.121	1.35 ±0.124	0.033 *	0.101	0.016 *
	NO3 soil (g/kg)	0.790 ±0.117	0.638 ±0.198	0.572 ±0.214	0.685 ±0.199	0.133	0.295	0.197
	NH4 soil (g/kg)	0.059 ±0.006	0.044 ±0.013	0.045 ±0.020	0.050 ±0.007	0.179	0.136	0.164

Table B.3: Averages and standard deviations of biomass and nutrient levels of the various soybean organs and soil nitrogen levels studied at maturity (R8 stage) under grazing and fertilisation treatments. Results of contrast test is exposed with "A" and "B" for pasture modalities and "$\triangle$" and "$\bigtriangledown$" for fertilisation modalities.

R8	Variable	Grazed		Non-grazed		P-values		
		Systemic	Conventional	Systemic	Conventional	Pasture	Fertilisation	Interaction
Plant	Pod number	$83.6^{A} \pm 18.6$	71.2 ±24.8	$66.7^{B} \pm 19.9$	68.0 ±18.0	0.004 **	0.036 *	0.102
	Pod biomass (g)	$35.1^{A\triangle} \pm 6.32$	$29.5^{\bigtriangledown} \pm 7.50$	$29.8^{B} \pm 7.61$	31.1 ±7.83	0.022 *	0.018 *	0.037 *
	Vegetative biomass (g)	32.9 ±5.59	31.4 ±5.60	29.9 ±8.28	33.3 ±7.81	0.139	0.091	0.262
	Total biomass (g)	$71.5^{A} \pm 15.4$	66.6 ±19.2	$60.1^{B} \pm 15.6$	64.4 ±14.5	0,031*	0.127	0.241
	N% pod	4.59 ±0.345	4.37 ±0.196	4.46 ±0.345	4.30 ±0.282	0.349	0.110	0.795
	P% pod	0.493 ±0.038	0.478 ±0.036	0.478 ±0.050	0.478 ±0.040	0.484	0.461	0.621
	K% pod	1.88 ±0.221	1.92 ±0.126	1.87 ±0.118	1.90 ±0.226	0.923	0.657	0.908
	N pod (g)	1.56 ±0.275	1.41 ±0.568	1.38 ±0.384	1.21 ±0.336	0.332	0.413	0.935
	P pod (g)	0.167 ±0.025	0.155 ±0.065	0.147 ±0.040	0.134 ±0.037	0.311	0.533	0.985
	K pod (g)	0.642 ±0.137	0.610 ±0.218	0.571 ±0.114	0.539 ±0.173	0.368	0.690	0.997
	N% leaves + stem	$0.976^{A} \pm 0.136$	0.924 ±0.108	$0.820^{B} \pm 0.185$	0.897 ±0.112	0.006 **	0.364	0.108
	P% leaves + stem	0.219 ±0.039	0.262 ±0.056	0.204 ±0.097	0.233 ±0.065	0.586	0.116	0.716
	K% leaf + stem	$0.672^{B} \pm 0.339$	1.03 ±0.352	$1.02^{A} \pm 0.389$	0.887 ±0.317	0.016 *	0.012 *	0.016 *
	N leaves + stem (g)	0.488 ±0.156	0.429 ±0.156	0.370 ±0.120	0.371 ±0.098	0.408	0.702	0.476
	P leaves + stem (g)	0.074 ±0.033	0.086 ±0.034	0.060 ±0.028	0.074 ±0.026	0.267	0.388	0.884
	K leaves + stem (g)	0.225 ±0.156	0.328 ±0.148	0.315 ±0.155	0.294 ±0.154	0.203	0.146	0.218
	N plant (g)	2.05 ±0.398	1.84 ±0.714	1.75 ±0.486	1.58 ±0.422	0.251	0.422	0.881
	P plant (g)	0.242 ±0.046	0.241 ±0.088	0.207 ±0.059	0.208 ±0.055	0.238	0.976	0.958
	K plant (g)	0.867 ±0.237	0.938 ±0.308	0.886 ±0.249	0.834 ±0.298	0.895	0.616	0.540
Soil	N Total soil (g/kg)	1.28 ±0.108	1.37 ±0.213	1.32 ±0.061	1.31 ±0.117	0.743	0.379	0.520
	NO3 soil (g/kg)	0.731 ±0.181	0.673 ±0.172	0.467 ±0.171	0.661 ±0.206	0.070	0.599	0.162
	NH4 soil (g/kg)	0.052 ±0.011	0.043 ±0.012	0.035 ±0.011	0.044 ±0.012	0.057.	0.303	0.143

Table B.4: Average and standard deviation of weight of thousand grains and total soybean yield according to the grazing and fertilisation treatment.

Yield	Variable	Grazed		Non-grazed		P-values		
		Systemic	Conventional	Systemic	Conventional	Pasture	Fertilisation	Interaction
	Thousand grain weight (g)	167 ±8.60	169 ±8.80	172 ±10.1	172 ±7.83	0.083.	0.579	0.576
	Productivity (kg/ha)	5979 ±691	5841 ±753	5833 ±711	5490 ±1017	0.650	0.668	0.651

Table B.5: Averages and standard deviations of LAI by combination of factors at each stage.

Stage	Grazed		Non-grazed		P-values		
	Systemic	**Conventional**	**Systemic**	**Conventional**	**Pasture**	**Fertilisation**	**Interaction**
12-march (R5,5)	4.50 ±0.508	4.60 ±0.587	4.79 ±0.462	4.64 ±0.488	0.234	0.676	0.466
22-march (R6)	$4.07^{A} \pm 0.296$	3.92 ±0.312	$3.71^{B} \pm 0.342$	3.73 ±0.306	0.0004***	0.137	0.226
27-march (R6+5D)	$3.42^{A} \pm 0.250$	$3.25^{A} \pm 0.278$	$2.76^{B} \pm 0.348$	$2.75^{B} \pm 0.364$	$3.93 * 10^{-10}$***	0.06	0.226

Table B.6: Averages and standard deviations of photosynthetic rate (µmol CO2/m^2/s) by combination of factors at each stage.

Stage	Grazed		Non-grazed		P-values		
	Systemic	**Conventional**	**Systemic**	**Conventional**	**Pasture**	**Fertilisation**	**Interaction**
06-march (R5,4)	26.0 ±1.95	25.2 ±1.50	25.2 ±2.07	25.1 ±3.63	0.3751	0.3420	0.5591
23-march (R6)	$24.0^{A\triangle} \pm 1.94$	$20.9^{\triangledown} \pm 3.68$	$20.5^{B} \pm 2.78$	19.8 ±3.35	0.004**	0.01*	0.156
28-march (R6+5D)	$20.1^{A} \pm 2.15$	$18.8^{A} \pm 2.64$	$15.2^{B} \pm 2.27$	$14.2^{B} \pm 2.14$	$1.91 * 10^{-9}$***	0.104	0.761
03-april (R7)	$15.6^{A} \pm 2.05$	$15.5^{A} \pm 2.60$	$11.3^{B} \pm 1.39$	$11.1^{B} \pm 1.14$	$1.19 * 10^{-10}$***	0.895	0.938

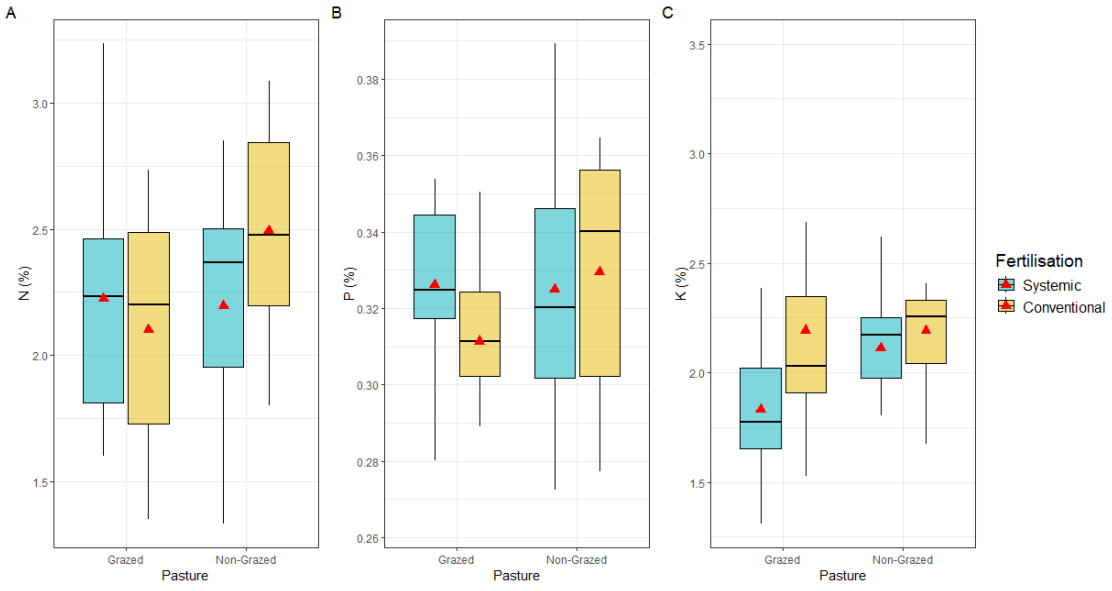

Figure 35: Boxplots of leaf + stem nutrients (%) per plant at R1 stage by factor combination with the representation of the average (△).

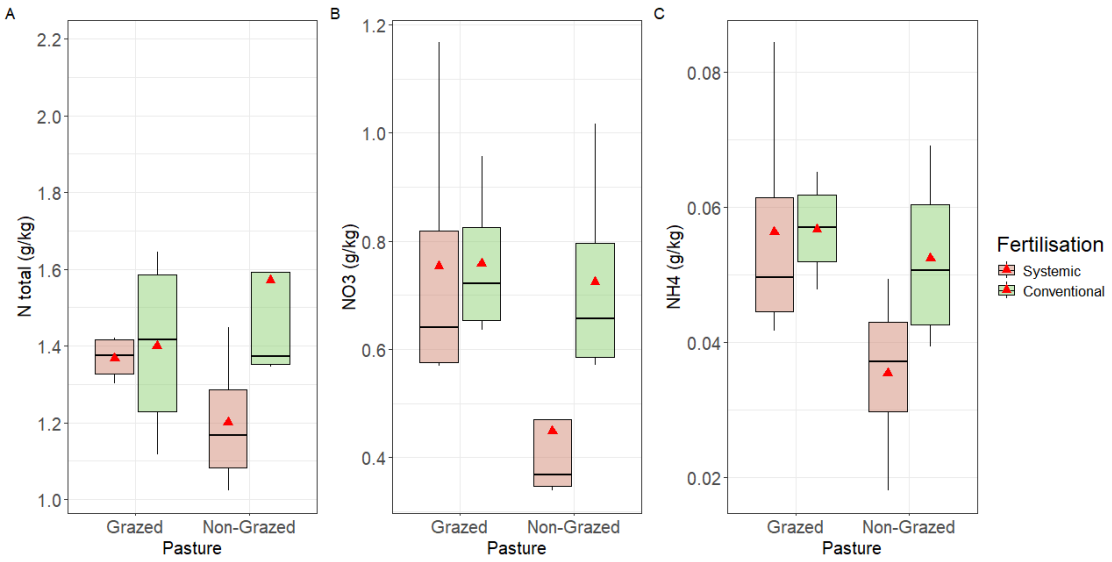

Figure 36: Boxplots of N total in the soil (g/kg) at R1 stage by factor combination with the representation of the average △.

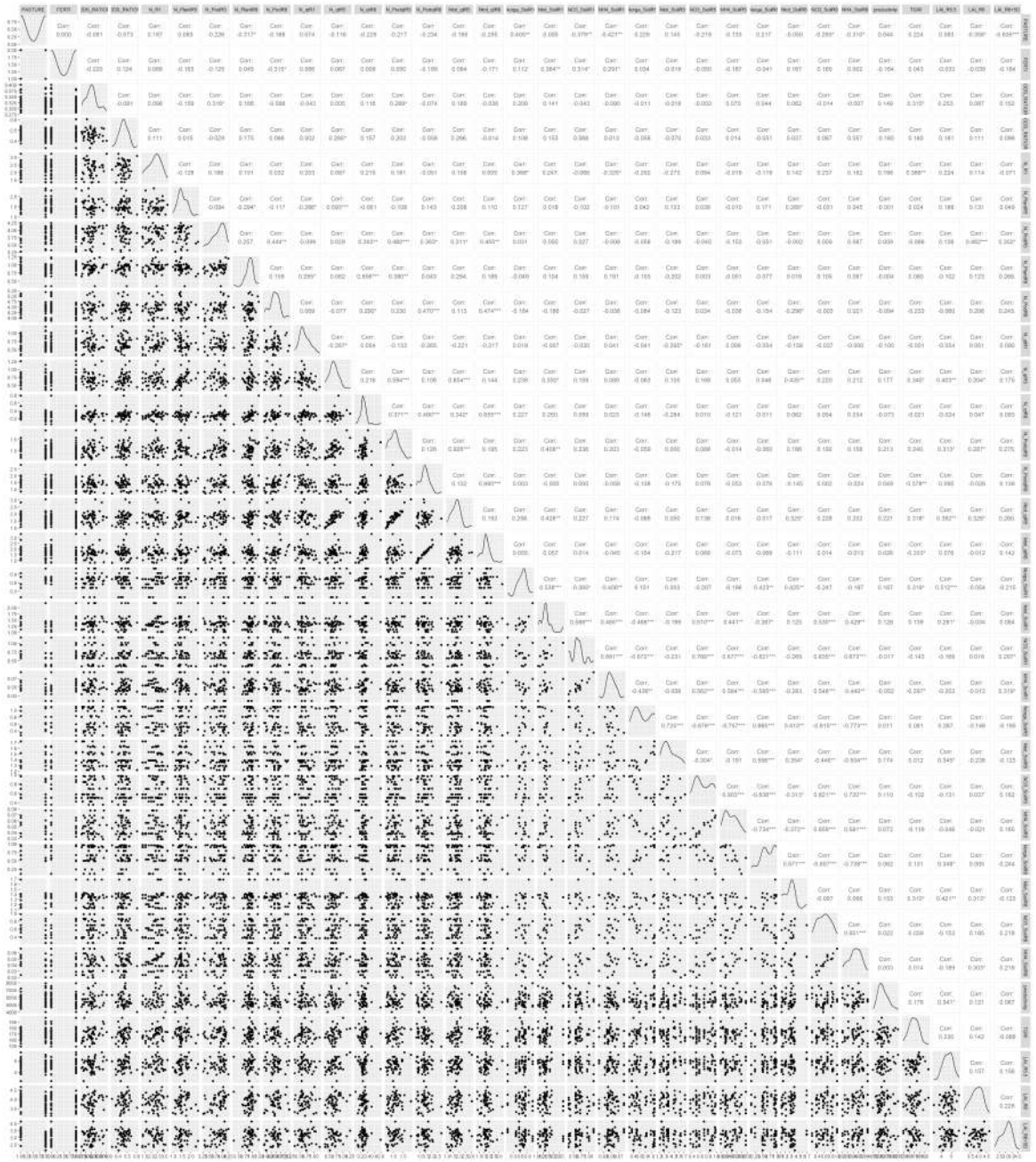

Figure 37: GGpairs general.

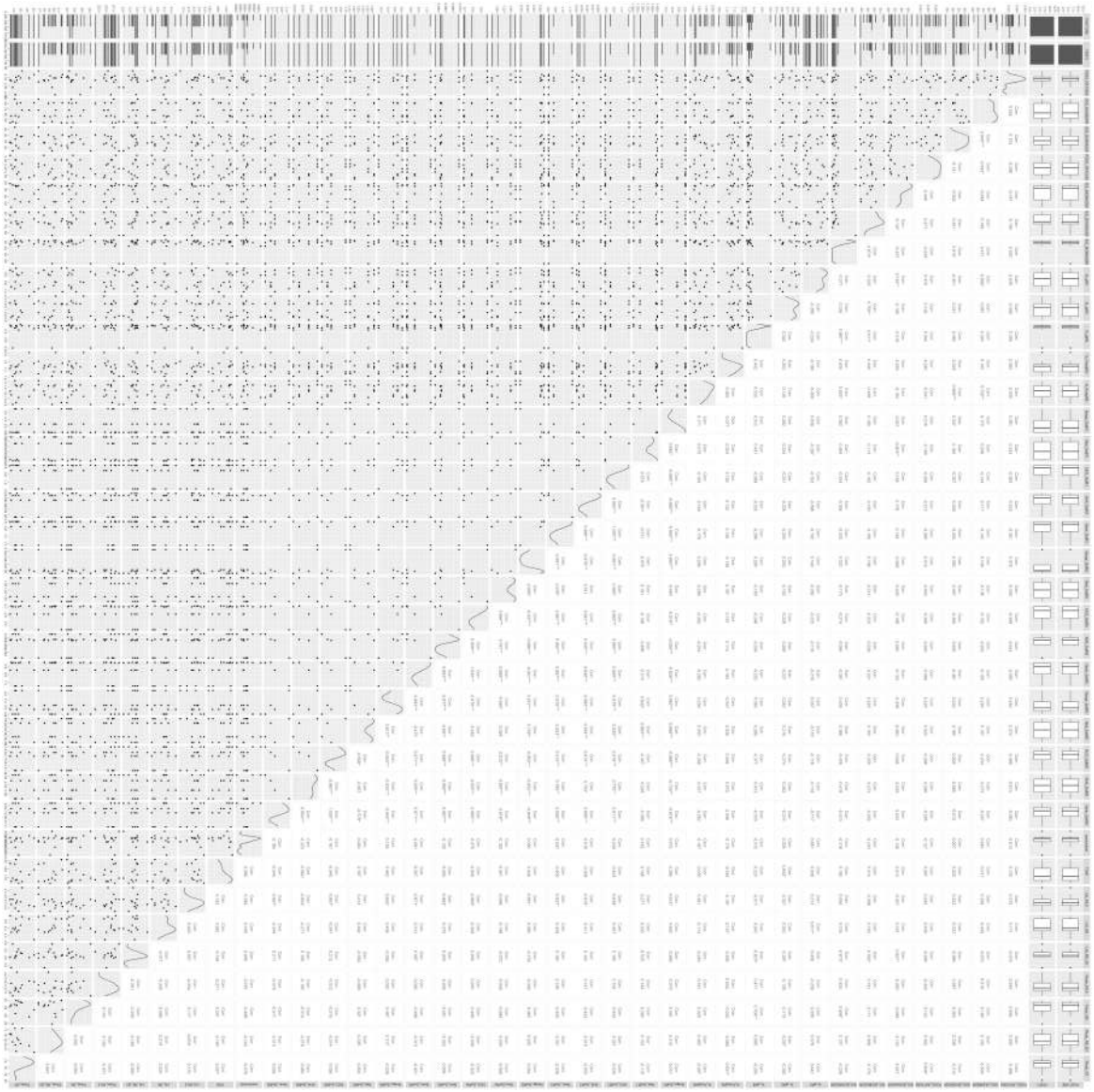

Figure 38: GGpairs of the Grazed-systemic combination.

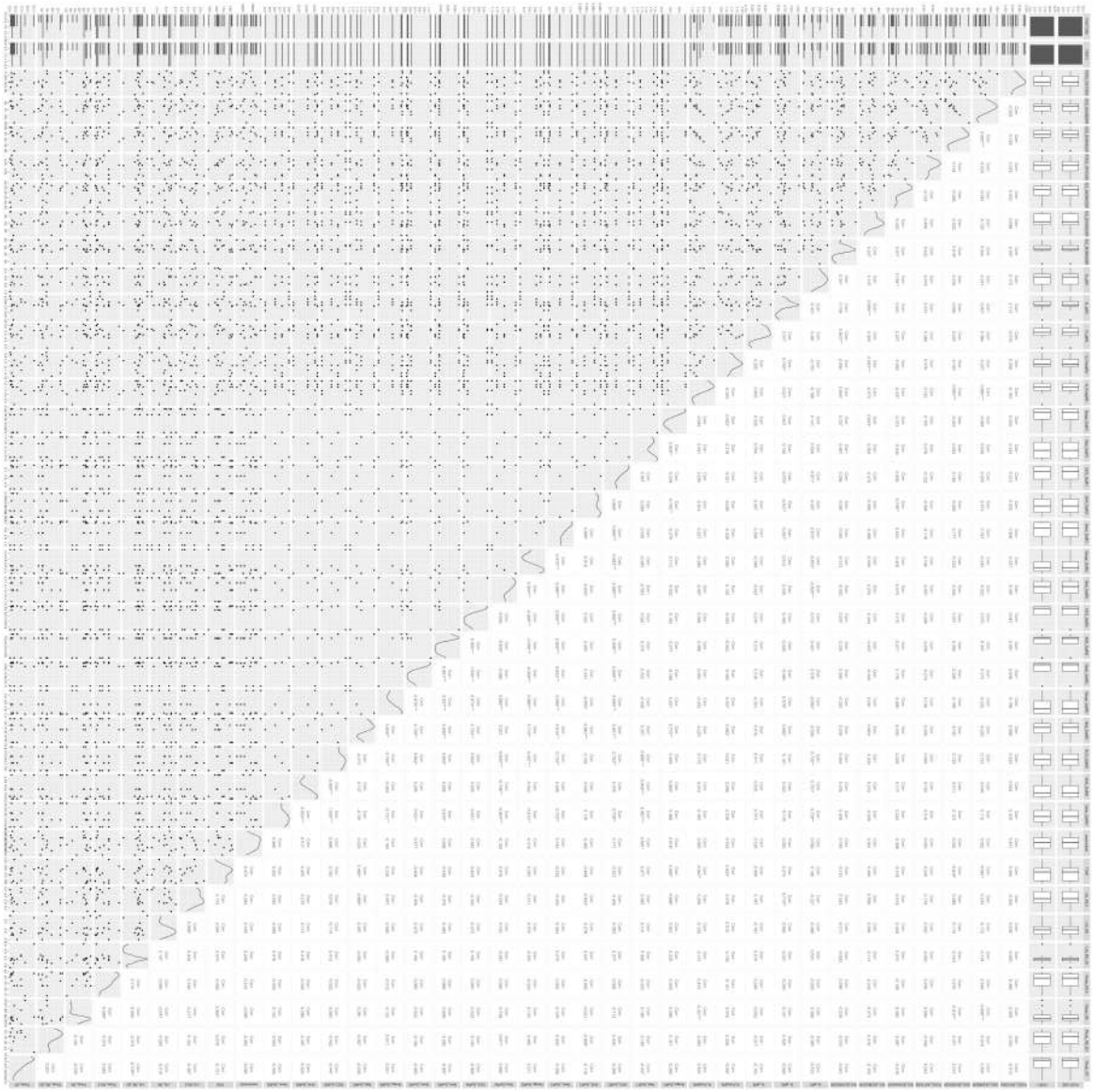

Figure 39: GGpairs of the Grazed-conventional combination.

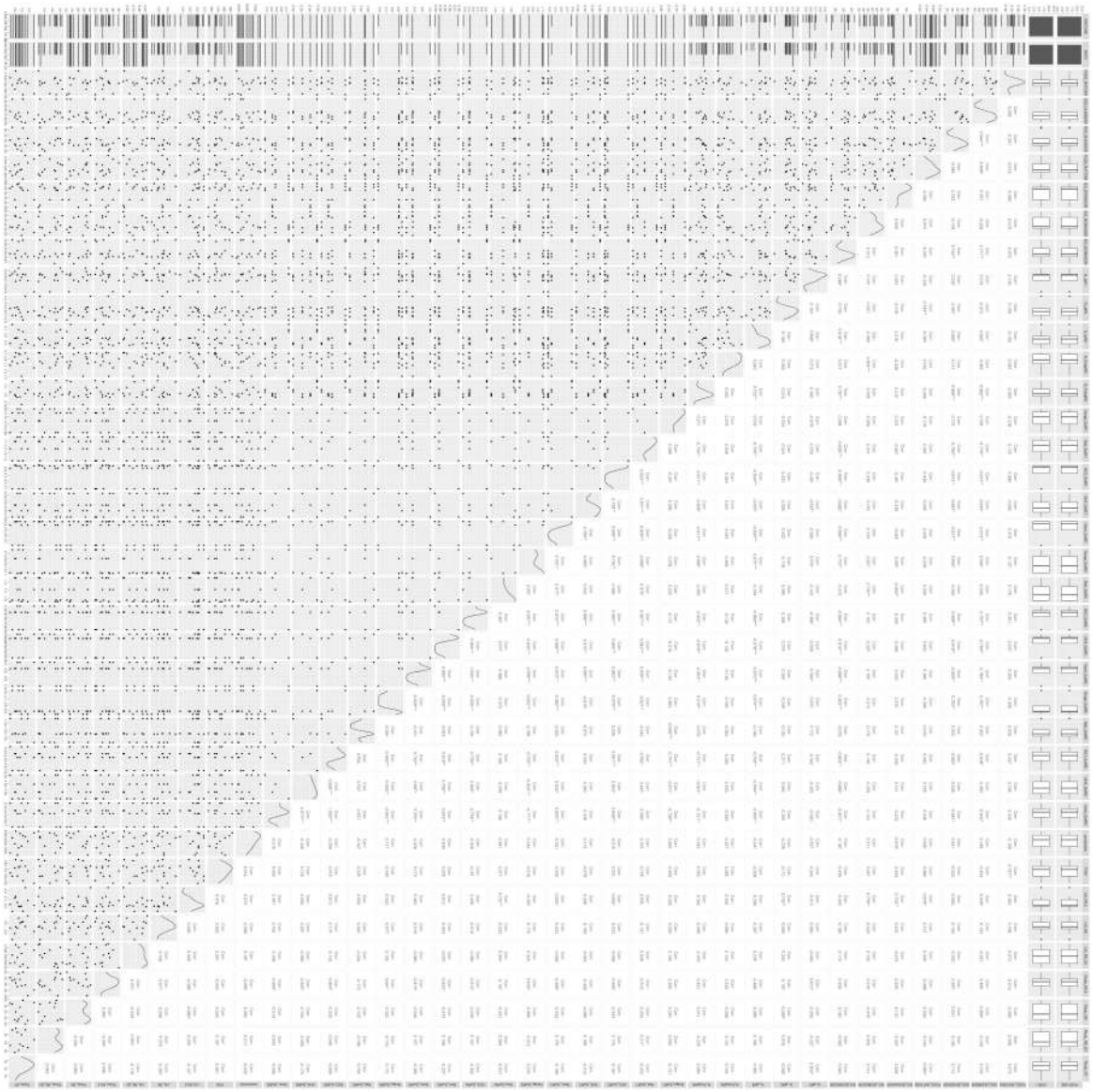

Figure 40: GGpairs of the Non grazed-systemic combination.

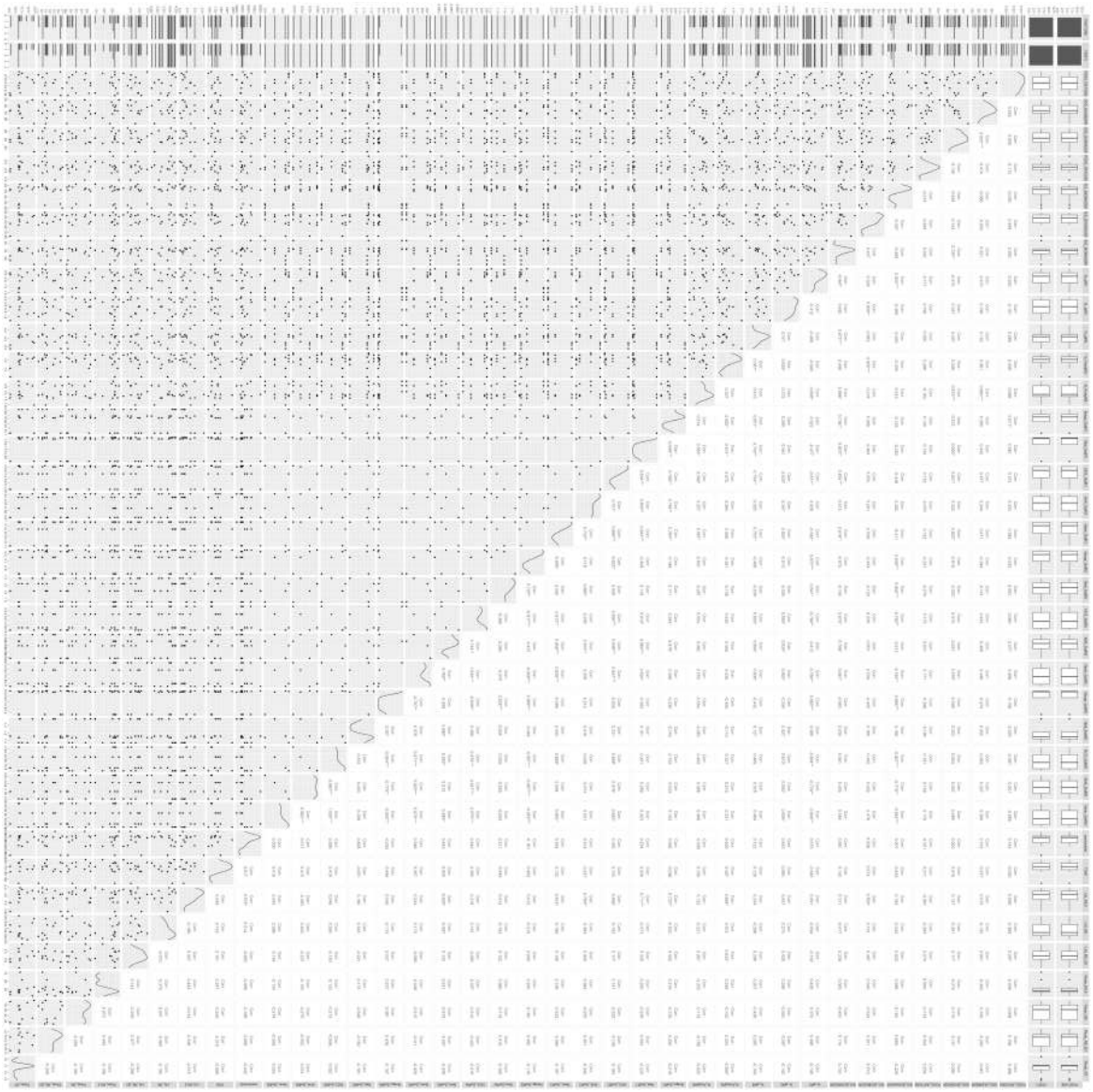

Figure 41: GGpairs of the Non grazed-conventional combination

Table B.7: Table of Pearson correlation coefficients between P and K levels in the plant at R1 stage and pod nitrogen quantity at the R8 stage.

Variable	PASTURE	FERTI	estimate	statistic	p.value	parameter	conf.low	conf.high	method
P plant (g) R1	Non-Grazed	Conventional	-0.553	-2.10	0.0622.	10	-0.855	0.0306	Pearson's
K plant (g) R1	Non-Grazed	Conventional	-0.574	-2.22	0.0508.	10	-0.864	-0.000710	Pearson's

Table B.8: Table of the relative contribution of each explanatory variable to class separation in the LDA model.

Variable	**LD1**	**LD2**	**LD3**
Root biomass R1	-0.154	0.079	-0.160
Veg biomass R1	-0.057	0.130	0.250
Veg biomass R5	-0.179	0.330	-0.112
Biomass/pod R5.5	-0.107	-0.382	0.008
Veg biomass R8	-0.140	0.123	0.045
Biomass/pod R8	-0.047	0.106	-0.205
N veg tissue R1	0.032	0.373	0.360
N veg tissue R5.5	-0.124	0.157	-0.015
N veg tissue R8	-0.264	0.169	0.095
N pod R5.5	-0.237	0.169	-0.002
N pod R8	-0.277	-0.213	0.114
N total R5.5	-0.211	0.183	-0.008
N total R8	-0.301	-0.146	0.121
TGW	0.252	-0.021	-0.032
Productivity	0.039	-0.301	0.037
LAI_R5.5	0.119	-0.246	-0.203
LAI_R6	-0.419	0.184	0.177
LAI_R6_5D	-0.705	-0.108	0.027
Photo_R5.4	-0.118	-0.165	0.366
Photo_R6	-0.461	0.061	0.314
Photo_R6_5D	-0.734	-0.216	-0.166
Photo_R7	-0.725	-0.022	-0.355