University of São Paulo
“Luiz de Queiroz” College of Agriculture

Dynamics of nitrogen partitioning in a historical set of soybean cultivars

Letícia Pacheco Inoue

Dissertation presented to obtain the degree of Master in Science. Area: Crop Science

Piracicaba
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1. Produtividade 2. *Glycine max 3.* Eficiência da utilização do nitrogênio 4. Índice de colheita de nitrogênio I. Título

DEDICATION

To my grandmother, Dinorah (in memorian), who taught me to be a strong and courageous person;

To all the professionals who work with science;

For everyone who, in some way,
contributed to the development
of this study

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(The opinions, hypotheses and conclusions or recommendations expressed in this material are the responsibility of the authors and do not necessarily reflect the views of CAPES, FEALQ and CNPq);

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The most important thing is the ability to tell apart
the impossible and the possible.
If it's simply difficult, as long as we have
the strong will and tenacity to go through it,
we'll make it;
-Philip Chesterfield

SUMÁRIO

RESUMO

Dinâmica da partição de nitrogênio em uma série histórica de cultivares de soja

Na cultura da soja o nitrogênio (N) é um dos nutrientes mais assimilados, no entanto esse elemento ainda é pouco explorado em relação às mudanças ocorridas na espécie durante o melhoramento e seleção de cultivares. Assim, o objetivo deste estudo foi avaliar como o melhoramento da soja no Brasil nos últimos 50 anos impactou o teor de N da soja, a partição de N em grãos e a concentração de proteína de grãos. Foram realizados dois experimentos, um de campo em duas safras (Experimento I) e outro em casa de vegetação (Experimento II). O Experimento I foi conduzido durante dois anos, as safras 2016/2017 e 2017/2018 e o Experimento II em uma safra. Em ambos os experimentos foi utilizado um delineamento em blocos aleatorizados. Foram obtidas sementes de cultivares representativas lançadas no período 1965 - 2016, sendo que para o Experimento I e II foram utilizadas 26 e 25 cultivares, respectivamente. No Experimento I as plantas foram amostradas durante a granação (R_5) e no ponto de colheita (R_8), enquanto no Experimento II foram amostrados quando a planta estava com 4 nós vegetativos (V_4), no florescimento (R_1), no período de fixação de vagens (R_3), além de R_5 e R_8. Durante o crescimento da cultura foram avaliados o teor e acúmulo de N na massa seca, a eficiência de uso de N e a partição de N. Para o experimento I o acúmulo de N aumentou ao longo do ano de lançamento dos cultivares (YOR) na biomassa da planta e nos grãos enquanto o N residual (remanescente na palhada) reduziu. O teor de N decresceu nos grãos, biomassa e resíduo ao longo do YOR. Verificou-se maior eficiência de utilização de N nas cultivares modernas em relação as mais antigas, e a partição de N na planta mostrou uma relação negativa entre o rátio do índice de colheita de nitrogênio (NHI) e índice de colheita (HI) com o YOR. Para o experimento II a massa seca apresentou uma tendência positiva para os grãos e biomassa ao longo do YOR e negativa para o resíduo. O teor de N reduziu ao longo do YOR nos grãos e no resíduo e aumentou levemente na biomassa. A eficiência de utilização de N aumentou e a partição propiciou uma relação negativa entre o rátio de NHI:HI e o YOR. Com isso, concluiu-se que houve um aumento no teor de N nas cultivares mais modernas, mas ocorreu um aumento mais intenso no teor de massa seca, o que causou uma diluição na quantidade de N total presente na planta, uma maior eficiência na utilização de N e uma menor relação entre o rátio NHI:HI e o YOR.

Palavras-chave: Produtividade; *Glycine max*; Eficiência da utilização do nitrogênio; Índice de colheita de nitrogênio

ABSTRACT

Dynamics of nitrogen partitioning in a historical series of soybean cultivars

In the soybean crop, nitrogen (N) is one of the most important nutrients. However, this element has been little explored in relation to changes that occurred in the species during the improvement and selection of cultivars. Thus, the aim of this study was to evaluate how soybean breeding in Brazil in the last 50 years has impacted soybean N accumulation, N partitioning to grains, and grain protein concentration. Two experiments were carried out, one in the field in two growing seasons (Experiment I) and the other in a greenhouse (Experiment II). Experiment I was conducted for two years, the 2016/2017 and 2017/2018 growing seasons, and Experiment II in one growing season. In both experiments a randomized block design was used. Seeds of representative cultivars launched in the period between 1965 and 2016 were obtained, and for Experiments I and II, 26 and 25 cultivars were used, respectively. In Experiment I the plants were sampled during the beginning of grains formation (R5) and at harvest (R8), while in Experiment II they were sampled when the plant was with 4 vegetative nodes (V4), at flowering (R1), in the beginning of pod formation (R3), in addition to R5 and R8. During crop growth, N accumulation in biomass, N concentration, N utilization efficiency, and N partitioning were evaluated. For Experiment I, N accumulation increased throughout the year of release of the cultivars (YOR) in plant and grain biomass while the residual N (remaining in the straw) decreased. The N concentration decreased in grains, biomass and residue along the YOR. There was a greater efficiency of N utilization in modern cultivars compared to older ones, and the ratio of nitrogen harvest index (NHI) to harvest index (HI) decreased with the YOR. For Experiment II, dry mass showed a positive trend for grains and biomass along the YOR and negative for the residue, the N accumulation showed the same trend as Experiment I but it was non-significant. The N concentration decreased along the YOR in grains and residue and increased slightly in biomass. The N utilization efficiency increased and there a negative relationship between the NHI:HI ratio and the YOR. Thus, it was concluded that there was an increase in the grain N content of more modern cultivars, but there was a more intense increase in the grain dry mass, which caused a dilution in the amount of total N present especially in the grain, a greater efficiency in use. of N and a smaller ratio between the NHI: HI and the YOR ratio.

Keywords: Productivity; *Glycine max*; N utilization efficiency; Nitrogen harvest index

FIGURE LIST

TABLE LIST

1. INTRODUCTION

Soybean (*Glycine max* (L.) Merr) is an important crop for the Brazilian agribusiness and is one of the main vegetable sources of protein and oil, which is used for human consumption, animal feed and industrial products (WILSON, 2008). During the 2020 growing season, 135 million tons of soybeans were produced in Brazil, which represents a 101% increase relative to the 1976/1977 growing season (CONAB, 2021). Of the world total production, in the 2020 season about 37% of the soybean produced came from Brazil (EMBRAPA, 2021). The world population is expected increase by about 30% until 2050, reaching around 10 billion people (UNITED NATIONS, 2015), which will increase the demand for agricultural production by 70% (RAY *et al.*, 2013). Meeting this demand is estimated to require a constant productivity increase of 2.4% per year (ESPE *et al.*, 2015).

Cultivation of high-yielding soybeans demands a large amount of nitrogen (N) to support healthy plant growth and produce high-protein soybean seeds (KRAPP, 2015). Salvagiotti *et al.*, (2008) revealed that soybean grain yield increases by 13 kg per each increase of one kg of plant N uptake on average. A high N content in soybean plants is required for optimal functioning of the photosynthetic apparatus (SRIVASTAVA *et al.*, 2006), since N is part of chlorophyll, the light-capturing pigment found in plant leaves (CIOMPI *et al.*, 1996; EVANS and SEEMANN, 1989). In addition, N simulates the activity and synthesis of Ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco), the enzyme responsible for CO_2 fixation. Nitrogen is also a component of amino acids, vitamins, nucleic acids, and other macromolecules needed throughout the plant (DECHEN and NATCHIGALL, 2007).

The N taken up by soybeans comes in part from the soil inorganic N pool and in part from the atmosphere by biological N fixation (BNF) (TAMAGNO *et al.*, 2018). Soybean roots take up nitrate (NO_3^-) and ammonium (NH_4^+), which are supplied through soil organic N mineralization. Another portion of N accumulated in soybean biomass comes from BNF, which comes from the transformation of atmospheric N (N_2) into plant-useable N forms (ROBERTSON and VITOUSEK, 2009). In particular, the diazotrophic bacteria *Bradyrhizobium japonicum* and *B. elkanii* form nodules on soybean roots, derive energy from the plant, and supply the plant with ureides, NH_4^+, and other N forms during growth (EMBRAPA, 2013). Biological N fixation contributes on average, 55% of the total N accumulated in the biomass, but there is a large range of BNF contribution of 0-94% among different varieties and environments (CIAMPITTI and SALVAGIOTTI, 2018).

Nitrogen is mobile in the plant and, therefore, can be remobilized from source tissues to new and forming tissues or physiological drains, which reinforces crop growth and development (TAIZ *et al.*, 2017). During reproductive growth, which includes flowering, fruiting, and seed formation, N moves from leaves to other plant components and particularly to the developing seeds (PAL and SAXENA, 1976; SINCLAIR and WIT, 1975). More than 60% of leaf N content can be remobilized to the grain (BENDER *et al.*, 2015). The N remobilization during grain filling may represent around 90% of the content in the grains, while the remainder comes from new N uptake(TAMAGNO *et al*., 2017). It is estimated that for each 1,000 kg of grain dry matter produced, about 80 kg of N are absorbed by the plant, about 60 kg of N are exported to the grains, and only 20 kg of N remain as crop residue (HUNGRIA and MENDES, 2015). A typical soybean crop in Brazil produces 3,500 kg ha^{-1}, so that 210 kg N ha^{-1} are harvested and 70 kg N ha^{-1} are returned to the soil (EMBRAPA, 2021).

In a historical set of soybean cultivars from Brazil, the number of seeds per area, the number of seeds per pod, the number of pods per plant, and the 100-seed weight were all positive in relation to the year of release (TODESCHINI *et al*., 2019). These findings are in agreement with studies carried out in the United States and China, which also showed that soybean yield increased along with cultivar year of release (ROWNTREE *et al*., 2013; JIN *et al*., 2011; LONG, 2013). Along with seed yield, whole plant dry matter has increased, though less than seed yield (TAMAGNO *et al.*, 2020). Despite higher yields and N removal per unit area in modern soybean cultivars (UMBURANAS, 2019; ORTEZ *et al*., 2018), some authors have observed a reduction in the grain protein (and N) concentration in the United States (RINCKER *et al*., 2014; ROWNTREE *et al.*, 2013; WILCOX *et al*., 1979) and in Brazil (TODESCHINI et al., 2019).

The reduction in grain protein may be explained by a dilution effect, in which modern soybean plants accumulate more dry matter but do not take up proportionally more N than older cultivars, leading to an overall decline of N concentration in plant tissues with year of release (CREGAN and YAKLICH, 1986). This trend would likely be reflected in a greater N utilization efficiency (NUtE) of modern cultivars, which is the ratio of seed yield to whole plant N uptake (CONGREVES *et al.*, 2021). It is also possible that the allocation of dry matter to grain has increased more than the allocation of N to grain (CREGAN and YAKLICH, 1986). The relative importance of these two mechanisms in explaining grain yield and protein concentrations is uncertain.

The decline in soybean seed N concentration is of concern because profitable marketing and use of soybeans depends on meeting minimum protein meal concentrations (46 to 48%) (PÍPOLO and MANDARINO, 2016). Another factor to be taken into account is the partial balance of N, which represents the return of N to the soil from the straw (the aerial part of the plant discounting the grains). It is important to understand the quantity of N returned to the soil to avoid long-term declines in soil N stocks (CIAMPITTI and SALVAGIOTTI, 2018). In order to maintain seed protein concentrations while increasing yield in the future and sustain soil N stocks, N partitioning dynamics in soybean plants needs to be better understood (SALVAGIOTTI *et al.*, 2008) especially in relation to Brazilian cultivars. A better understanding of soybean N partitioning can improve crop modelling and management strategies (VAN ROEKEL and PURCELL, 2014). The objective of this study was to determine how soybean breeding in Brazil over the last 50 years has impacted soybean N accumulation, N partitioning to grain, and grain protein content.

1.1. Nitrogen: Metabolic function, importance and uptake

Nitrogen (N) is an essential element and plays a fundamental role in plants (NOVOA and LOOMTS, 1981). Nitrogen is found in proteins, which act as catalysts in metabolic pathways, as structural elements of the cytoplasm and membranes, and as carriers.in transport functions. Nitrogen is also a component of nucleic acids, which allow for the translation, coding and storage of genetic information (NOVOA and LOOMTS, 1981). In the photosynthetic process, N is present in the chlorophyll molecule, the proteins found in tylacoid menbrane and RuBisCo (EVANS and SEEMANN, 1989). In addition, N participates in vegetative growth such as leaves, stem, root system and reproductive growth, and in the composition of flowers (LEGHARI *et al.*, 2016).

Plants take up N primarily as dissolved inorgnic ions – nitrate (NO_3^-) and ammonium (NH_4^+). The concentrations of these ions in soil solution are generally low (NOVOA and LOOMTS, 1981). However, inorganic N is continually resupplied as soil organic matter (SOM) decomposes and mineralizes C and N (WEBER and MIELNICZUK, 2009).

Nitrogen absorption by plants is done first in the free space of the roots (space of the cell wall) and then through the membranes in the plant cells themselves. The transport in the membrane occurs in both ‘passive’ and ‘active’ forms. The ‘passive’ form depends on the permeability of the membrane, occurring through diffusion in the electromechanical gradient without the need for the use of metabolic energy. In contrast, the ‘active’ form uses metabolic energy through "carriers" composed of protein units (NOVOA and LOOMTS, 1981).

While ammonium is directly available to participate in the plant’s metabolic activities, nitrate needs to be reduced ammonium through a series of reduction steps. Cytosolic nitrate reductase (GS1) reduces nitrate to nitrite and the chloroplast enzyme nitrite reductase (GS2) reduces nitrite (NO_2^-) to ammonium (NH_4^+) (OHYAMA, 2010). The reduction steps are carried out primarily in the aboveground part of the plant. In this new form, N becomes mobile in the plant and can participate in the metabolism of stem, leaves, flowers, pods, and grains (KRAPP, 2015). The re-assimilation of ammonium released by the amino acid catabolism, during leaf senescence, for example, is also influenced by GS1 (LOTHIER *et al*., 2011).

1.2. Nitrogen partitioning in soybean

According to the globally adopted soybean phenological scale (FEHR and CAVINESS, 1977), the stages of soybean development can be divided into two phases, Vegetative (V) and Reproductive (R). The vegetative phase can be subdivided into emergence (V_E), cotyledon (V_C), then numerically from V_1 to Vn, where the ‘n’ represents the number of vegetative nodes, until leaf growth stops in plants with a determinant growth habit, and until flowering begins in the plants with an indeterminant growth habit. The reproductive growth until maturity is represented from R_1 to R_8, as follows: R_1 indicates the beginning of flowering, R_2 full flowering, R_3 beginning of fruiting, R_4 full fruiting, R_5 beginning of seed formation, R_6 seeds formed with full grain (100%), R_7 physiological maturity, R_8 field maturity and natural leaf fall.

The productivity of fabaceous crops, such as soybeans, is highly affected by the absorption and partitioning of N between the vegetative and reproductive organs (WESTERMANN *et al.*, 1985). At the beginning of the reproductive period,, there is a decrease in the N concentration of stems, roots, and leaves as the plant translocate N to the reproductive organs (PAL and SAXENA, 1976). The ‘self-destruction’ hypothesis suggests that the intense demand for N by the grain filling generates a rapid depletion of the other plant organs (SINCLAIR and DE WIT, 1975). The N remobilization during grain filling may represent 30 to 100% of the content in the grains. The remobilization values from the vegetative part to the grains are higher than 60% (ZEIHER *et al*., 1982).

2. OBJECTIVES

2.1. General

To evaluate the dynamics of N and its partitioning in the plant (soybean N accumulation, N partitioning to grain, and grain protein content) among a sequence of soybean cultivars released over the last 50 years in Brazil.

2.2. Specific

a) To evaluate N partitioning in a historical series of soybean cultivars throughout the growth cycle
b) To estimate the N utilization efficiency (NUtE) in plants grown in the field and in pots; and
c) To estimate, under field conditions, the remaining N content in the soybean crop residues after harvest of the different cultivars.

3. MATERIAL AND METHODS

This study included a field experiment carried out in two growing seasons (Experiment I), and also a pot experiment carried out in one growing season (Experiment II). The cultivars used in both experiments represent a historical set of Brazilian cultivars, released from 1965 to 2016, contemplating cultivars with determinant (D), indeterminant (I), and half-determinant (HD) growth habits (Table 1).

Table 1. List of soybean cultivars used in the field experiments (I) and in pot experiment (II).

Year of release	Cultivar	Growth habit	Field experiment	Pot experiment
1965	Davis	D	X	X
1966	Santa Rosa	D	X	X
1968	Campos Gerais	D	X	X
1973	IAS3	D	X	X
1974	Paraná Marrom	D	X	X
1974	Paraná	D	X	X
1976	Viçoja	D	X	X
1979	BR4	D	X	X
1988	FT Abyara	D	X	X
1985	FT-11 Alvorada	D	X	X
1985	Ocepar 3 - Primavera	I	X	X
1985	Ocepar 4-Iguaçu	D	X	X
1985	BR16	D	X	X
1989	FT Cometa	I	X	X
1991	BR36	D	X	X
1998	MG/BR 46 (Conquista)	D	■	X
1998	Embrapa 48	D	X	X
2003	CD 206	D	X	X
2005	BRS 245 RR	D	■	X
2007	BRS 282	D	X	X
2007	BRS 284	I	X	X
2008	BMX Potência	I	X	X
2011	AFS 110	I	X	X
2011	TMG 7262 RR	I	X	X
2012	Nidera 5909	HD	X	X
2014	MSOY 5892 IPRO	HD	X	■
2015	BMX Ícone	I	X	
2016	BMX Garra	I	X	

D, Determinant growth habit; I, Indeterminant growth habit; and HD, Half determinant growth habit

3.1. Experiment I

Experiment I was carried out in the field at Guarapuava-PR, Brazil (25° 23'S, 51° 27'O, 1029 m altitude), with a climate classified as Cfb - Köppen (ALVARES *et al.*, 2013) and a soil classified as a Ferralsol (WRB/FAO, 2007) or Latossolo Bruno (SANTOS *et al.*, 2018). The experimental design consisted of a randomized complete block design, with 26 different soybean cultivars (Table 1) and three replicates. Each plot consisted of four rows spaced 0.45 m and 5 m in length (5 x 1.8 m). The field experiment was conducted during the 2016/2017 and 2017/2018 growing seasons. The preceding crops prior to the 2016/2017 season were potatoes (*Solanum tuberosum*) in summer season and black oat (*Avena strigosa*) in winter season, whereas prior to the 2017/2018 season were maize (*Zea Mays*) in summer and black oats (*Avena strigosa*) in winter season. Both fields were managed without tillage. Chemical analysis was made with ten soil samples, collected with an auger at depths of 0-0.20 m and 0.20-0.40 m (Table 2).

Table 2. Soil chemical analysis of the areas used in Experiment I for the 2016/2017 and 2017/2018 growing seasons. Soils were sampled to depths of 0-20 cm and 20-40 cm. Guarapuava, Paraná, Brazil.

Season	Depth	pH	O.M.	P resin	K	Ca	Mg	H+Al	Al	CEC	V%	m%	S-SO$_4$
	m	CaCl$_2$	g/dm^3	mg/dm^3		mmol$_c$ dm^{-3}				mmol$_c$ dm^{-3}			mg/dm^3
2016/2017	0-0.2	5.3	37	35	1.2	36	12	34	0	82	59	0	11
	0.2-0.4	4.5	32	5	1.2	21	10	80	1	112	29	3	56
2017/2018	0-0.2	4.7	39	39	3.5	33	13	64	2	114	44	4	12
	0.2-0.4	4.8	27	4	1.2	19	13	58	0	36	36	0	29

O.M.: Organic Matter; CEC: Cation Exchange capacity; V%: Base saturation; m%: Al saturation

Seeding was performed on November 4 in both seasons. Basic fertilization was made, which consisted of 80 kg P_2O_5 ha^{-1}, 71 kg Ca ha^{-1}, 35 kg ha^{-1} S (single superphosphate) and 70 kg ha^{-1} K_2O (potassium chloride). The seeding rate was 28 seeds ha^{-1} and row spacing 0.25 m. Also, before sowing, seeds were treated with pyraclostrobin [25 g L^{-1}], methyl thiophanate [225 g L^{-1}] and fipronil [250 g L^{-1}] at the rate of 2 mL per kg of seeds, and inoculated with *Bradyrhizobium japonicum*, using 2.4 g turfous inoculant per kg of seeds containing around 5 billion viable cells per gram of inoculant.

Five plants of each plot (each cultivar), were collected at the R_5 stage and partitioned in stem, leaf and pod in the two seasons (2016/2017 and 2017/2018). Five plants of each treatment were also collected at the R_8 stage, which were also partitioned in a stem, leaf, pod and grain in the 2016/2017 and 2017/2018 seasons (Table 4). After separating the plant tissues, the samples were subjected to drying in a forced aeration oven at 65ºC until constant mass.

Meteorological data collected in a meteorological station located about 100 m away from the planting area were quite similar in both years, with average temperatures of 20.6 and 20.3 ºC, respectively; 2584 and 2537 MJº m^{-2} for the cumulative solar radiation; and the cumulative rainfall was 823 and 984 mm, respectively. There were no problems of water stress in this season as reported in Umburanas (2019), and for the southern region of Brazil, 800 mm of rainfall are considered sufficient for a soybean crop (ZANON *et al*., 2016).

3.2. Experiment II

The Experiment II was carried out in semi-controlled environment in Piracicaba-SP, Brazil (22° 42′S, 47° 38′O, 546 m altitude) with a climate classified as Cwa - Köppen (ALVARES *et al*., 2013). The pot experiment received controlled irrigation and took place at average daily temperature of 22 to 24ºC.

The experimental design was a completely randomized block design with 25 cultivars (Table 1) and with four replicate blocks. Soybean plants were grown in pots of 8.3 dm^3, with sand and vermiculite substrate, in a 2:1 volume ratio. A chemical analysis was performed after mixing vermiculite with sand, so that was possible to analyze the ideal nutrient content to be applied for the plants (Table 3).

Table 3. Chemical analysis of the substrate (Sand with vermiculite 2:1).

pH	O.M.	P	K	Ca	Mg	H+Al	Al	CEC	V%
$CaCl_2$	g dm^{-3}	mg dm^{-3}			mmolc dm^{-3}			mmolc dm^{-3}	
5.4	5	4	2	9	43	12	1	66	81
$CaCl_2$	Mg	K	Cu	Fe	Zn	Mn	B	m%	S-SO_4
	%				mg dm^{-3}				mg dm^{-3}
14.3	68.3	3.2	0.3	10	0.5	3	0.13	2	6

O.M.: Organic Matter; CEC: Cation Exchange capacity; V%: Base saturation; m%: Al saturation

After chemical analysis, an initial fertilization was carried out with 350 mg dm^{-3} of Ca; 36 mg dm^{-3} of P, 20 mg dm^{-3} of S, 76 mg dm^{-3} of K, 0.17 mg dm^{-3} of B, 0.01 mg $plant^{-1}$ of Co and 0.083 mg dm^{-3} of Mo The sources of this fertilization were single superphosphate, potassium phosphate monobasic, elemental boron and solutions of cobalt and molybdenum. Before sowing, the pots were cleaned and sterilized with 80% alcohol and 10% sodium hypochlorite and washed with deionized water.

On December 22, 2016 five seeds were sown per pot. Seeds were inoculated with *Bradyrhizobium japonicum* (SEMIA 5079) and *Bradyrhizobium elkanii* (SEMIA 5019) (EMBRAPA, 2020). Because the substrate did not contain N and there was no N fertilizer added, BNF was the only source of N for this experiment.

Thinning was done at the V_c-V_1 stage (FEHR and CAVINESS, 1977) to maintain two plants per pot. The pots were placed under full sun throughout the cycle; insects and diseases were adequately controlled.

At the V_4, R_1, R_3, R_5 and R_8 stages, destructive collections of the plants were carried out. The plants were partitioned into root, stem leaf, pod and grain (Table 4), and subjected to drying in a forced aeration oven at 75ºC until constant mass.

Table 4. Evaluation attributes in Experiments I and II.

<table>
<tr><th colspan="3">Experiment I</th></tr>
<tr><td>Season</td><td>Stage*</td><td>Plant Part</td></tr>
<tr><td rowspan="2">16/17</td><td>R_5</td><td>Stem, leaf, Pod</td></tr>
<tr><td>R_8</td><td>Grain, Leaf, Stem, Pod</td></tr>
<tr><td rowspan="2">17/18</td><td>R_5</td><td>Stem, leaf, Pod</td></tr>
<tr><td>R_8</td><td>Grain, Leaf, Stem, Pod</td></tr>
<tr><th colspan="3">Experiment II</th></tr>
<tr><td colspan="2">Stage*</td><td>Plant Part</td></tr>
<tr><td colspan="2">V_4</td><td>Root, Leaf</td></tr>
<tr><td colspan="2">R_1</td><td>Root, Leaf, Stem</td></tr>
<tr><td colspan="2">R_3</td><td>Stem, Leaf, Pod</td></tr>
<tr><td colspan="2">R_5</td><td>Root, Leaf, Stem, Pod</td></tr>
<tr><td colspan="2">R_8</td><td>Stem, Pod, Grain</td></tr>
</table>

*Phenological stages were based on Fehr and Caviness (1977). For Experiment II, not all plant parts are listed for all growth stages because some samples were lost after grinding and before chemical analysis.

3.3. Total-N analysis

Plant samples from both experiments were milled until they reached a particle size of 0.1 mesh or 1.19 mm. Sub-samples were analyzed for total N concentration by the sulfuric digestion method followed by the spectrophotometer method with salicylic blue(WALINGA *et al.*, 1995).

First, a sample of 0.1 g was weighed into a test tube and digested after adding 0.4 g of the catalyst (1:3 copper sulphate and potassium sulphate - crushed into fine powder) and 2 ml of sulfuric acid (H_2SO_4). The tubes were brought to a heat block for approximately 4 hours, with the temperature gradually increasing, starting at 100ºC for 30 minutes, 200ºC for another 30 minutes, 300ºC for 1 hour, and 400ºC for 2 hours or until complete digestion with completely oxidized content, presenting a light green color. The digestion was diluted by first adding Milliq® water, until reaching the 50ml meniscus. Then, 1 ml of the diluted digestion was pipetted into a falcon tube and brought to 10ml using ultrapure water (Milli-q® water). This solution is now called 'aliquot'.

The salicylic blue method was used to analyze the plant digests (WALINGA *et al*., 1995). To do this, it was necessary to prepare solutions of salicylic acid ($C_7H_6O_3$) 5% (Solution A), Nitroprusside sodium ($Na_2[Fe\ (CN)_5NO]$) 0.1% (Solution B) and Sodium hypochlorite (NaOCl) 0.15% (Solution C) and standard $N\text{-}NH^{4+}$ solutions (0 to 1.25 mM).

For analysis, 1 ml of aliquot, 1 ml of solution A, 1 ml of the solution B, and 1 ml of solution C were pipetted in a 10 ml test tube, and supplemented with 6 ml of Milliq® water. Then, the sample was left to rest for 60 minutes to complete reaction, acquiring a more greenish color. A spectrophotometer at 697nm was used for readings. A standard curve with concentrations of $N\text{-}NH_4^+$ ranging from 0 to 1.25 mM was used to relate absorbances to solution concentrations. The solution concentrations were converted to plant tissue concentrations using the appropriate dilution factor and mass of the tissue sample.

3.4. Nitrogen Utilization Efficiency and Allocation Indices

To calculate the Nitrogen Utilization Efficiency (NUtE), equation (1) below was used (CONGREVES *et al*., 2021). This method is a simple expression of production, calculated in units of productivity, or yield (Y) by units of nutrient in the organ of interest or plant nitrogen (PN).

$$\mathrm{NUtE(kg/ha) = Y(kg/ha)/PN(kg[N]/ha)} \qquad (1)$$

To calculate the allocation indices, a correlation was made between the Nitrogen Harvest Index (NHI) to Harvest Index (HI) ratio with the years of release. Nitrogen Harvest Index was calculated as the ratio between N accumulation in grains per N accumulation in biomass and Harvest Index was calculated as the ration between the dry mass of the grains per dry mass of the biomass.

3.5. Statistical Analyses

The data were subjected to simple linear regression, where the year of release was the explanatory variable. Pearson's correlation calculation was also performed at a p value of 5% ($p < 0.05$), used to attribute statistical significance to the slope estimates. Both linear regression and Pearson correlation calculation were performed with all replications. The software used was Microsoft Excel®. Cultivars were also grouped by the Scott-Knott test (1974) at the level of 5% significance, using the software Sisvar- UFLA. Scott Knott's method was chosen to deal with a comparison of multiple means, that is, it is an effective method in comparing a high number of treatments, in addition it classifies the data into groups in a homogeneous way.

4. RESULTS

4.1. Experiment I

Total N accumulation and allocation in soybean plants by year of release

The regression lines showed an average N accumulation for grains of 105 kg N ha^{-1} in 1965 and 185 kg N ha^{-1} in 2016, corresponding to an annual increase of 1.6 kg N ha^{-1} averaged between the seasons (Figure 1A). Soybean total biomass N regression lines predicted an average of 233 kg N ha^{-1} in 1965 and 281 kg N ha^{-1} in 2010 averaged across the two seasons. This corresponded to an increase of 0.94 kg N ha^{-1} per year (Figure 1B). And for the straw, the average N accumulation was 128 kg N ha^{-1} in 1965 and 96 kg N ha^{-1} in 2010 with a decrease of 0.64 kg N ha^{-1} per year (Figure 1C).

Scott Knott's analysis separated the N accumulation values for total biomass in 2016/2017 and in 2017/2018 into four groups (Table 5). The analysis separated the N accumulation values for grain into five groups for 2016/2017 and nine for 2017/2018 (Table 5). And for the accumulation of N in the straw, there were five groups for 2016/2017 and for 2017/2018 (Table 5). For biomass and grain, the set of cultivars categorized as “A”, with the lowest N accumulation, were primarily released before 1990 in both years. However, for straw N accumulation, the cultivars categorized as “A” were released primarily after 1990. The set of cultivars in the categories with the highest N accumulation in biomass and grain were primarily released after 1990 while those categorized with the highest N accumulation in straw were released before 1998.

Cultivars were relatively consistent in terms of relative N accumulation in the two years. For example, Davis (1965) was in the lowest group in both years whereas BR16 (1985) and BR36 (1991) were in the highest group in both years for biomass N accumulation. For grain N accumulation, Davis (1965) and Santa Rosa (1996) were cultivars with low N and BRS 284 (2007) and BMX Ícone (2015) were cultivars with high N. And, for straw accumulation, Santa Rosa (1966) and FT Cometa (1989) were cultivars with low and high N, respectively.

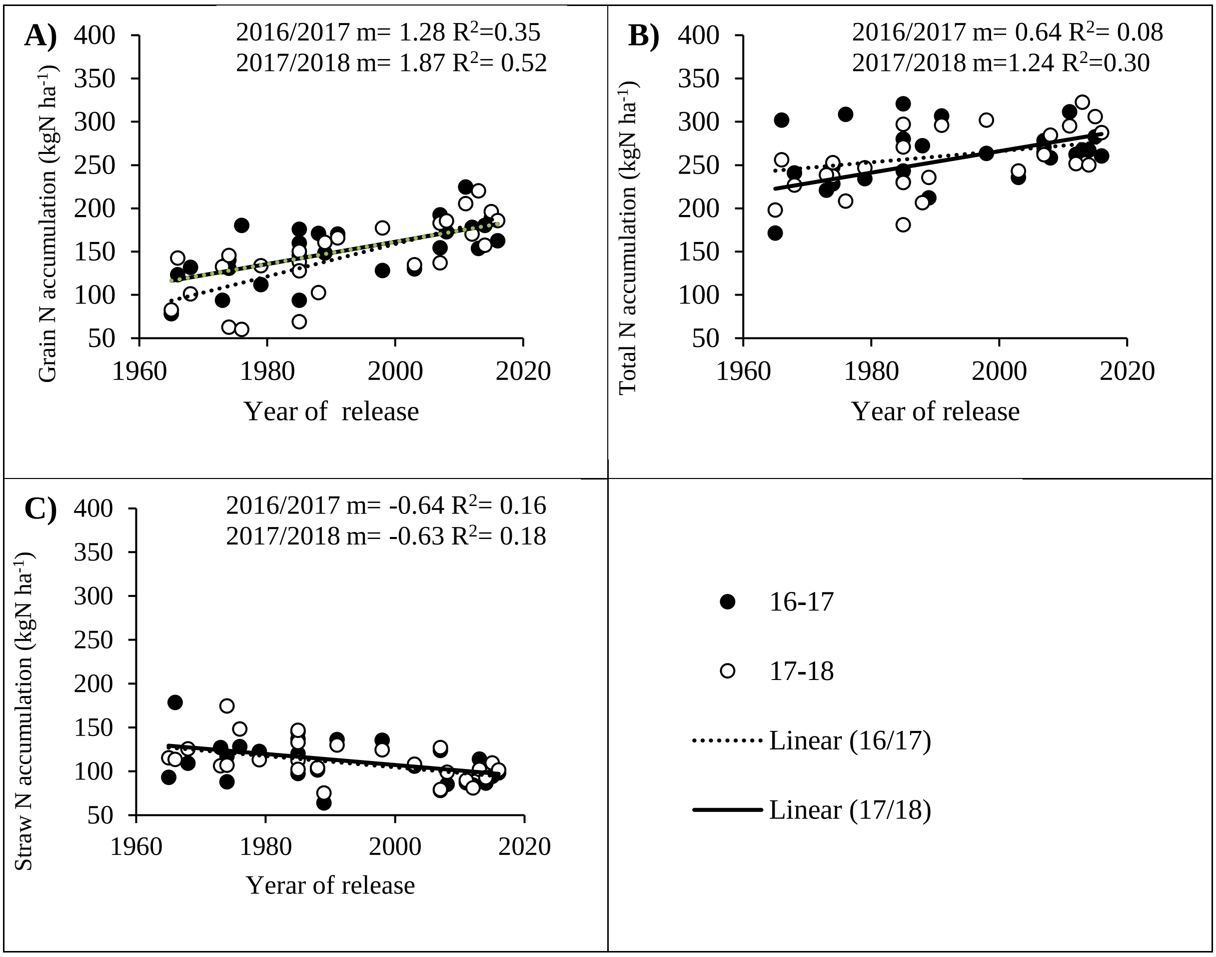

Figure 1. Total N accumulation found in A) Grains, B) Whole plant biomass and C) Straw correlated with the year of release of the cultivar in the stage R_8 from Experiment I. Plotted points are averages in order to facilitate visualization but regressions were performed with all replicates.

Table 5. N accumulation groups in total biomass, grains and straw, with respect to the year of development in the 2016/2017 and 2017/2018 growing seasons.

Year of release	Cultivar	Biomass accumulation (Kg[N] ha^{-1})											
		Grain		Total		Straw		Grain		Total		Straw	
		2016/2017						2017/2018					
1965	Davis	78	A	171	A	93	B	83	B	198	A	116	B
1966	Santa Rosa	124	B	302	D	179	E	63	A	237	B	174	E
1968	Campos Gerais	132	B	241	B	109	B	143	D	256	B	114	B
1973	IAS3	94	A	221	B	127	C	101	C	227	B	126	C
1974	Paraná Marrom	131	B	248	B	117	C	133	D	239	B	106	B
1974	Paraná	140	B	228	B	88	A	146	E	253	B	107	B
1976	Viçoja	180	D	309	D	128	C	60	A	208	A	148	D
1979	BR4	112	B	234	B	123	C	134	D	247	B	113	B
1988	FT Abyara	171	C	273	C	102	B	103	C	207	A	104	B
1985	FT-11 Alvorada	94	A	231	B	137	D	69	A	181	A	112	B
1985	Ocepar 3 - Primavera	146	C	243	B	98	B	139	D	271	C	133	C
1985	Ocepar 4-Iguaçu	160	C	280	C	120	C	128	D	230	B	102	B
1985	BR16	176	D	321	D	145	D	151	E	297	D	147	D
1989	FT Cometa	148	C	212	B	64	A	161	E	236	B	75	A
1991	BR36	171	C	307	D	136	D	166	F	296	D	130	C
1998	Embrapa 48	128	B	264	C	135	D	177	G	302	D	125	C
2003	CD 206	130	B	236	B	106	B	135	D	243	B	108	B
2007	BRS 282	155	C	278	C	124	C	137	D	265	C	127	C
2007	BRS 284	192	D	270	C	78	A	183	G	262	C	79	A
2008	BMX Potência	173	C	258	C	85	A	185	G	284	D	99	B
2011	AFS 110	225	E	312	D	87	A	205	H	295	D	90	A
2012	Nidera 5909	178	D	262	C	84	A	171	F	252	B	81	A
2013	TMG 7262 RR	154	C	268	C	114	C	220	I	323	D	102	B
2014	MSOY 5892 IPRO	181	D	267	C	87	A	157	E	250	B	93	A
2015	BMX Ícone	189	D	283	C	94	B	196	H	306	D	110	B
2016	BMX Garra	163	C	261	C	98	B	186	G	288	D	101	B
	Cultivar		***		***				***		***		
	CV%		12		9				6		6		

Uppercase letters in columns classify the averages by Scott Knott's test at 5% of significance.

Dry matter and N content

According to Umburanas, (2019), the soybean grain yield increased over year of release. Regression lines predicted that the grain average was 202 g m^{-2} in the 1965 cultivar and 394 g m^{-2} in the 2016 cultivar. The rate of yield increase was 3.9 g m^{-2} per year (Figure 2A). There was a significant decrease in soybean straw dry matter with year of release, the average showed 1168 g m^{-2} in 1965 and 852 m g^{-2} in 2016, with a rate of 6.2 g m^{-2} ($p<0.05$, Figure 2C), while the total biomass dry matter showed a non-significant change with year of release ($p>$ 0.05, Figure 2B).

Laboratory analyses showed that the N concentration in grains and straw represented in mg g^{-1} showed a decreasing trend in relation to the historical selection studied while the total biomass N concentration showed an increasing trend. Grain N content decreased in average from 52 mg g^{-1} to 47 mg g^{-1} at a rate of -0.10 mg g^{-1} per year (Figure 3A). The total biomass N content increased in average from 18 mg g^{-1} to 29 mg g^{-1} at a rate of 0.21 mg g^{-1} per year (Figure 3B). On the other hand, straw presented a milder decrease, which was not statistically significant ($p>0.05$, Figure 3C).

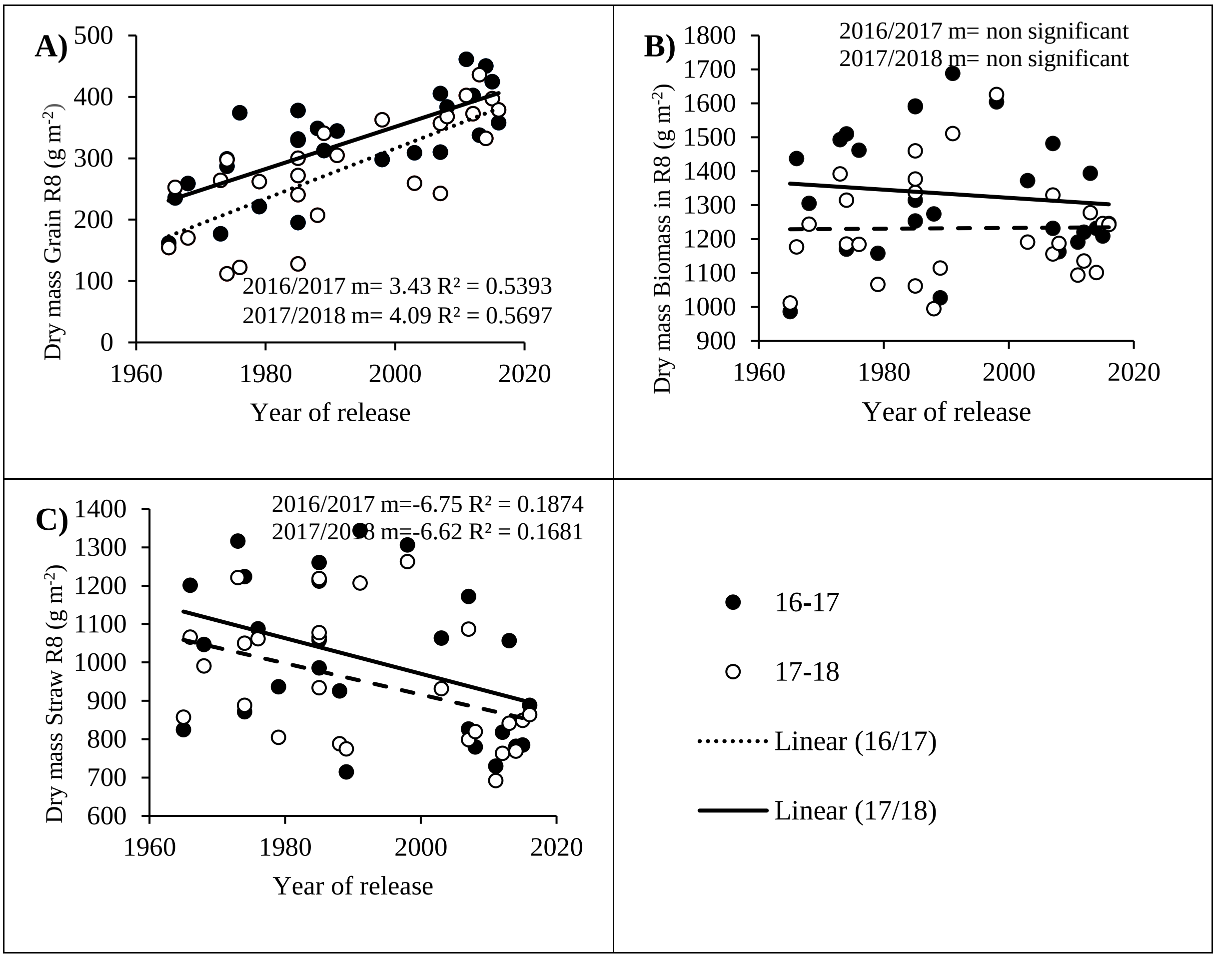

Figure 2. Mass values at stage R_8 compared to the year of release for A) Grains, B) Whole plant biomass, and C) Straw from Experiment I. Data collected from Umburanas (2019). Plotted points are averages in order to facilitate visualization but regressions were performed with all replicates

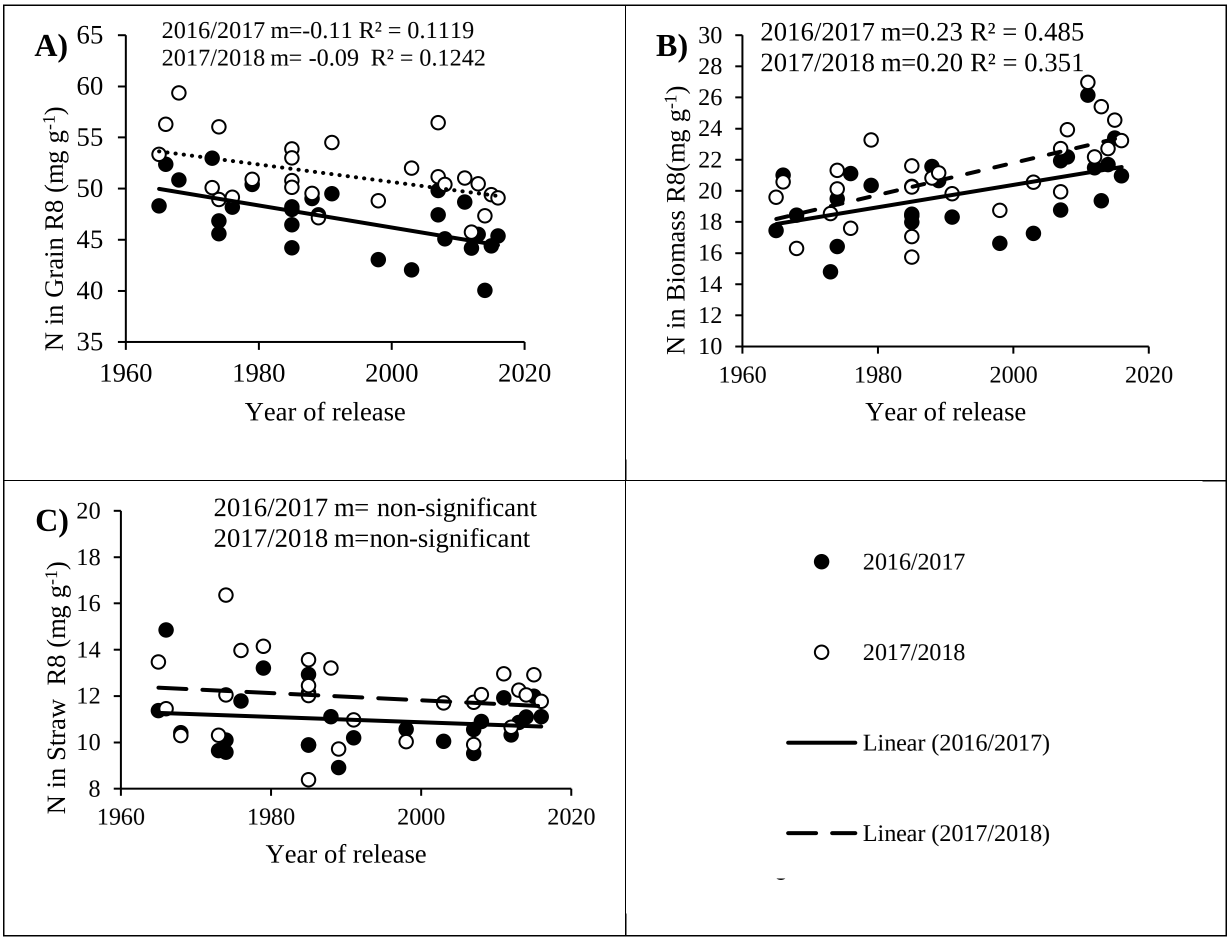

Figure 3. Values of N present per gram at stage R8 compared to the year of release for A) Grain, B) Whole plant biomass, and C) Straw from Experiment I. Plotted points are averages in order to facilitate visualization but regressions were performed with all replicates.

Nitrogen Utilization Efficiency (NUtE)

The soybeans regression lines showed an average of 9 to 14 kg grain per kg N uptake. In a general way, N utilization efficiency analysis showed an increasing trend compared to the year of release (Figure 4), in both growing seasons. As noted in other analyses, cultivars were relatively consistent over the two years. In this case, Santa Rosa (1966) was in the lowest group in both years, while MSOY 5892 IPRO (2014) was in the highest group in both years in regard to NUtE (Table 6).

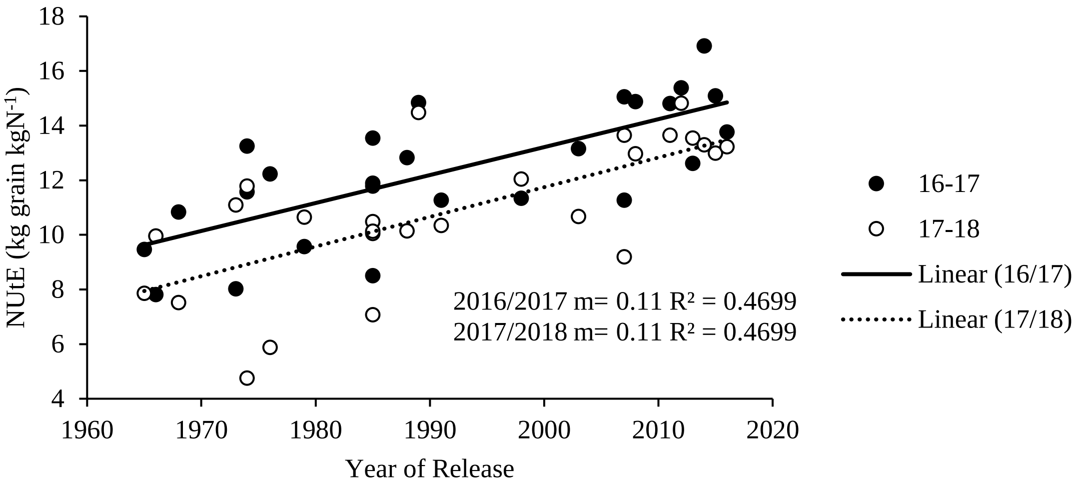

Figure 4. Nitrogen utilization efficiency (NUtE) in relation to the year of release from Experiment 1. Plotted points are averages in order to facilitate visualization but regressions were performed with all replicates.

Table 6. N utilization efficiency, with respect to the years of release in the 2016/2017 and 2017/2018 growing season.

Cultivar	Year of release	NUtE in 2016/2017 ($kg[N]^{-1}ha^{-1}$)	NUtE in 2017/2018 ($kg[N]^{-1}ha^{-1}$)
Davis	1965	9.5 B	7.9 C
Santa Rosa	1966	7.8 A	4.7 A
Campos Gerais	1968	10.8 C	9.95 D
IAS3	1973	8.0 A	7.5 C
Paraná Marrom	1974	11.6 C	11.1 E
Paraná	1974	13.2 D	11.8 E
Viçoja	1976	12.2 C	5.9 B
BR4	1979	9.6 B	10.7 D
FT Abyara	1988	12.8 D	10.2 D
FT-11 Alvorada	1985	8.5 A	7.1 C
Ocepar 3 - Primavera	1985	13.5 D	10.0 D
Ocepar 4-Iguaçu	1985	11.9 C	10.5 D
BR16	1985	11.8 C	10.1 D
FT Cometa	1989	14.8 E	14.5 G
BR36	1991	11.3 C	10.3 D
Embrapa 48	1998	11.3 C	12.1 E
CD 206	2003	13.1 D	10.7 D
BRS 282	2007	11.3 C	9.2 D
BRS 284	2007	15.0 E	13.6 F
BMX Potência	2008	14.9 E	13.0 F
AFS 110	2011	14.8 E	13.6 F
Nidera 5909	2012	15.4 E	14.8 G
TMG 7262 RR	2013	12.6 D	13.5 F
MSOY 5892 IPRO	2014	16.9 F	13.3 F
BMX Ícone	2015	15.1 E	13.0 F
BMX Garra	2016	13.8 D	13.2 F
Cultivar		***	***
CV%		7	6

Uppercase letters in columns classify the averages by Scott Knott's test at 5% of significance.

Nitrogen partitioning

Figure 5 illustrates the dynamics of N partitioning in the plant for each growth stage evaluated throughout the set of historical cultivars in the field study. Values are expressed as a percentage (%) in relation to the N accumulated throughout the plant. Of the total N uptake, an average of approximately 56% of the N was allocated to the grain at the R8 stage.

The Pearson analysis correlating the percentage of N in each organ with the year of release showed that at the stage R5, the stem showed a decreasing trend ($p<0.05$) in the 2016/2017 growing season, but not in 2017/2018. On the other hand, leaves showed a positive trend for both seasons, but showed significance only in the second growing season. For pods, a non-significant negative trend was observed for both seasons ($p > 0.05$, Figure 5A and B).

At stage R8, the stem, leaf and pod presented for both growing seasons a significant negative trend ($p < 0.05$). For the grains, there was a significant positive relationship with year of release for both seasons ($p < 0.05$, Figure 5C and D).

Having this in mind, the regression made between the NHI:HI ratio and the year of release showed a decrease from 2.8 mg g^{-1} 1965 to 2.0 mg g^{-1} 2012 (Figure 6).

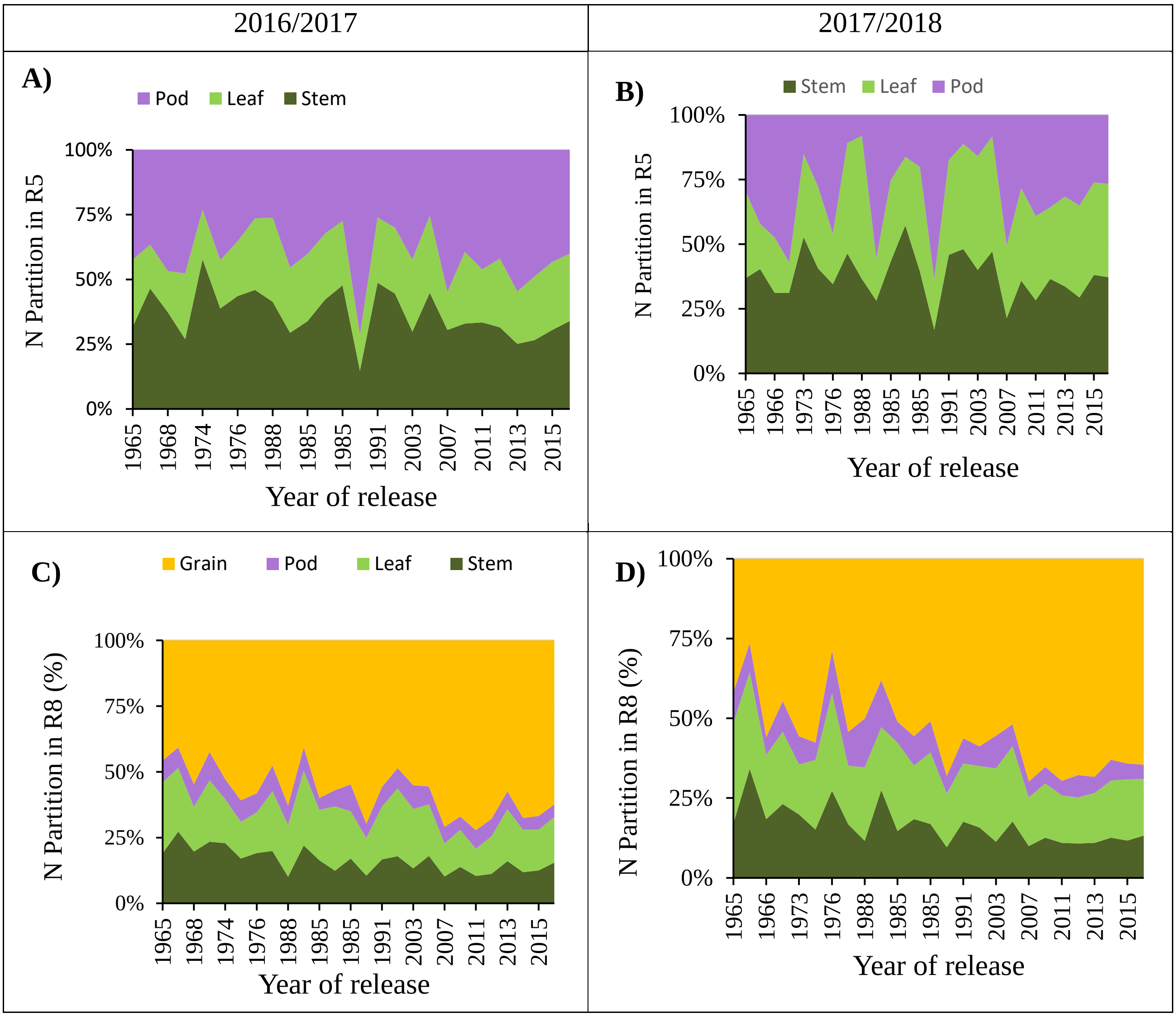

Figure 5. Dynamics of N partition between the plant organs at each growth stage compared with the year of release from Experiment I. In each growing season, 2016/2017 and 2017/2018. A) R_5 2016/2017 B) R_5 2017/2018 C) R_8 2016/2017 D) R_8 2017/2018.

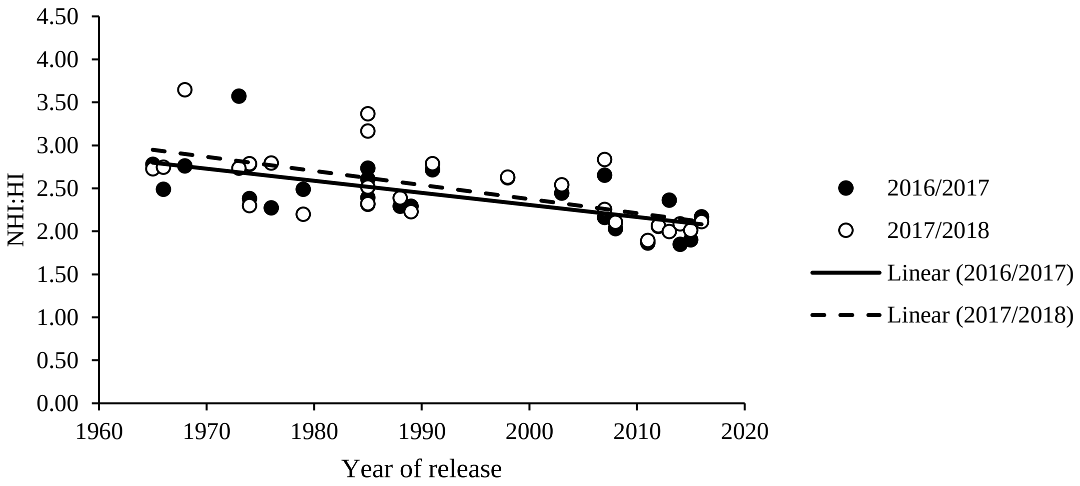

Figure 6. Correlation between the ratio of nitrogen harvest index (NHI) and harvest index (HI) with the year of release for Experiment I. Plotted points are averages in order to facilitate visualization but regressions were performed with all replicates

4.2. Experiment II

Total N accumulation and allocation in soybean plants by year of release

For some of the evaluated cultivars in this greenhouse study, samples were lost after dry mass was recorded but before N analysis could be performed. Therefore, the N concentrations of one replicate were used for the corresponding treatments in the other replicates to calculate the N accumulation.

There was non-significant linear relationship between total N accumulation and year of release for the greenhouse study ($p > 0.05$, Figure 7A). However, there was a significant quadratic relationship illustrating an increase in total N content between 1965 and 1990 and a decrease in total N content between 1990 and 2010 ($p < 0.05$, Figure 7A). This relationship was driven largely by the two cultivars circled in red in Figure 7A, AFS110 and Nidera 5909. These data points are considered possible outliers because the modern cultivars with low N accumulation in the greenhouse study had relatively high N accumulation in the complementary field study. There was also non-significant relationship between grain N accumulation or straw N accumulation and year of release ($p > 0.05$, Figure 7B and 7C). However, a negative association between straw N accumulation and year of release was visually observed

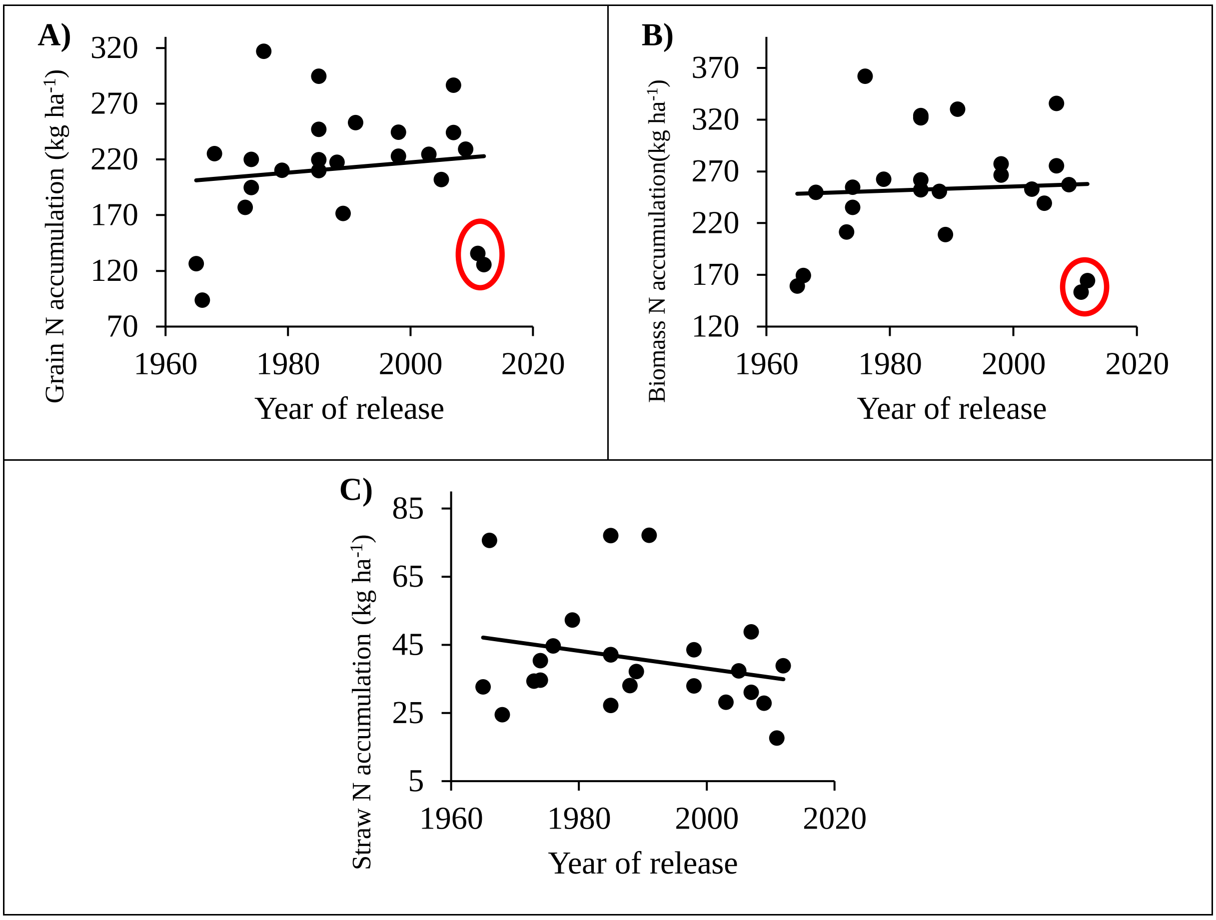

Figure 7. Total N accumulation in response to year of release of the cultivar in the stage R_8 from Experiment II. The linear regression did not show significance ($p > 0.05$), while the quadratic regression did ($p < 0.05$) in A) Grains and B) Biomass. Two points circled in red were largely responsible for the quadratic relationship. Plotted points are averages in order to facilitate visualization but regressions were performed with all replicates. The C) Straw graphics didn’t demonstrate significance ($p > 0.05$).

Scott Knott's analysis separated the cultivars into four groups for N accumulation in total biomass, six groups for N accumulation in grains and two groups for N accumulation in straw (Table 7). The highest value for biomass N accumulation is presented by the cultivars BMX Potência (2007), FT-11 Alvorada (1985), Ocepar 4-Iguaçu (1985) and BR36 (1991), to the lowest value we have the cultivars Davis (1965), Santa Rosa (1966), AFS 110 (2011) and Nidera 5909 (2012). For grain N accumulation, the highest values are presented by the cultivars BMX Potência (2007) and Viçoja (1976) and the lowest by the cultivar Santa Rosa (1966), while the highest mean value of N accumulation in the straw was found in cultivar BR36 (1991), while the lowest mean value was found in the cultivar AFS110 (2011).

Table 7. N accumulation values in total biomass, grains and straw, with respect to the year of development from Experiment II.

Year of release	Cultivar	Biomass N accumulation (Kg[N] ha^{-1})		
		Total	Grain	Residue
1965	Davis	159 A	127 B	33 A
1966	Santa Rosa	169 A	94 A	76 B
1968	Campos Gerais	250 C	225 D	25 A
1973	IAS3	212 B	177 C	34 A
1974	Paraná Marrom	235 C	195 D	40 A
1974	Paraná	255 C	220 D	35 A
1976	Viçoja	362 B	317 F	45 A
1979	BR4	263 C	210 D	52 A
1988	FT Abyara	251 C	218 D	33 A
1985	FT-11 Alvorada	324 D	247 E	77 B
1985	Ocepar 3 - Primavera	262 C	220 D	42 A
1985	Ocepar 4-Iguaçu	322 D	295 F	27 A
1985	BR16	252 C	210 D	42 A
1989	FT Cometa	209 B	172 C	37 A
1991	BR36	330 D	253 E	77 B
1998	Embrapa 48	277 C	244 E	33 A
1998	MG/BR 46 Conquista	267 C	223 D	44 A
2003	CD 206	253 C	225 D	28 A
2005	BRS 245 RR	239 C	202 D	37 A
2007	BRS 282	275 C	244 E	31 A
2009	BRS 284	257 C	229 D	28 A
2007	BMX Potência	336 D	287 F	49 A
2011	AFS 110	153 A	136 B	18 A
2012	Nidera 5909	164 A	126 B	39 A
	Cultivar	***	***	***
	CV%	9	9	36

Uppercase letters in columns classify the averages by Scott Knott's test at 5% of significance.

Soybean dry matter allocation and tissue N concentrations by year of release

According with Umburanas (2019) in the greenhouse study, the regression line had an average soybean grain yield ranging from 511 g m^{-2} the 1965 cultivar to 609 g m^{-2} in the 2012 cultivar (Figure 8A). The rate of yield increase was 2.08 kg ha^{-1} per year. The biomass production presented an increase from about 1065 g m^{-2} in 1965 to 1075 g m^{-2} in 2012, the rate of increase was 0.19 g m^{-2} per year. Straw dry matter decreased from 555 g m^{-2} in the cultivar released in 1965 to 466 g m^{-2} in the 2012 cultivar. With a rate of 1,89 kg ha^{-1} per year.

Laboratory analyses showed that the N concentration in grains and in the straw represented in mg g^{-1} showed non-significant change with year of release ($p > 0.05$, Figure 9). However, we must take into account that the outliers were also considered in this calculation and may mask some real value.

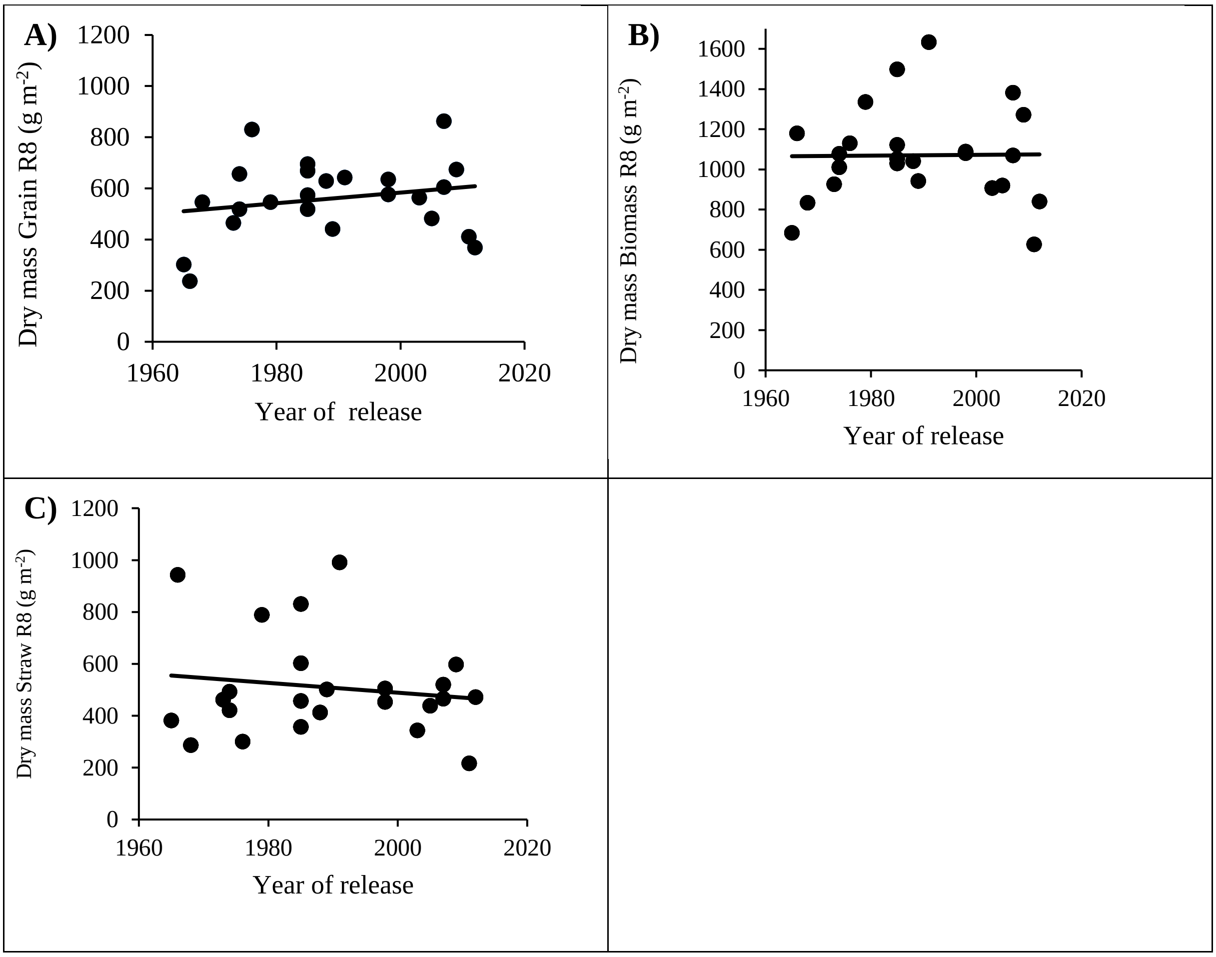

Figure 8. Dry mass values at stage R_8 compared to the year of release for A) Grain, B) Whole plant biomass, and C) Straw from Experiment II. Data were collected from Umburanas (2019). Plotted points are averages in order to facilitate visualization but regressions were performed with all replicates

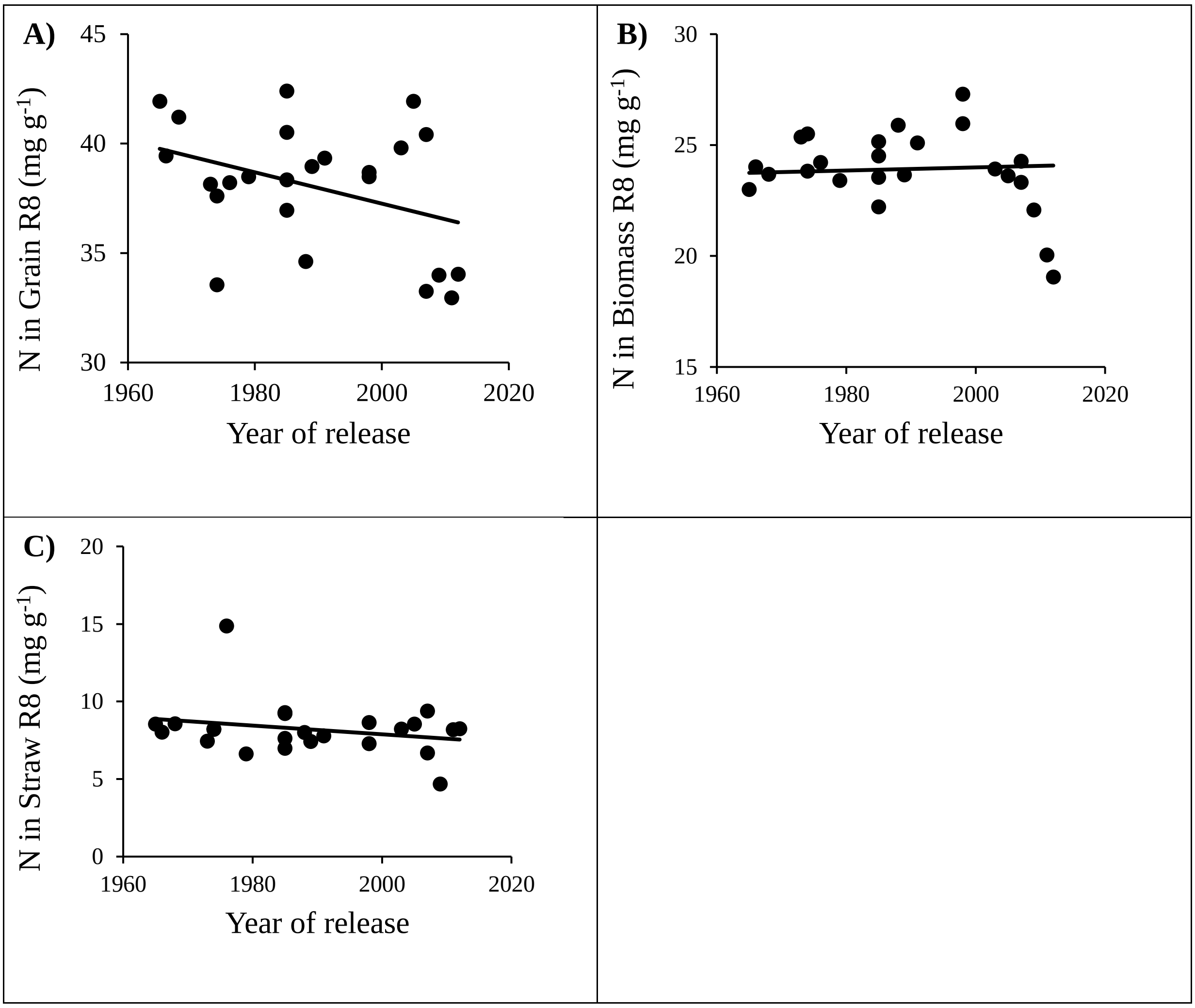

Figure 9. Values of N present per gram at stage R_8 compared to the year of release for A) Grain, B) Whole plant biomass, and C) Straw from Experiment II. Plotted points are averages in order to facilitate visualization but regressions were performed with all replicates.

Nitrogen Utilization Efficiency (NUtE)

Nitrogen Utilization Efficiency increased with year of release for the greenhouse study, from an average of 20 kg grain kg^{-1} N in the earliest cultivars to 24 kg grain kg^{-1} N in the most modern cultivars The rate of increase in NUtE was 0,08 kg grain kg^{-1} N per year. In this experiment the regression was also significant at a significance level of 5%.

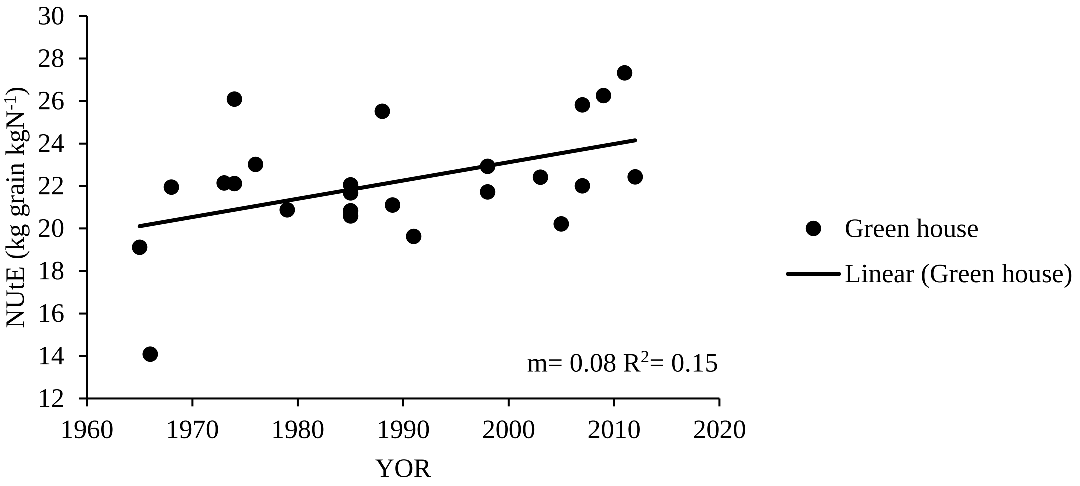

Figure 10. N utilization efficiency in relation to cultivar year of release from Experiment II. Plotted points are averages in order to facilitate visualization but regressions were performed with all replicates

The Scott Knott test divided the NUtE levels assessed in Experiment II into three groups, with values presenting significance at 5% (Table 8). The cultivars with the highest average NUtE content were AFS 110 (2011), FT Abyara (1988), Paraná (1974), BRS 284 (2009) and BMX Potência (2007) and the cultivar with the lowest average NUtE content was Santa Rosa (1966). The result found in the Scott Knott analysis from the greenhouse were very similar to that found in the field. This reaffirms the increase in NUtE in the most modern cultivars in relation to the oldest cultivars.

Table 8. N utilization efficiency, with respect to the years of release.

Cultivar	Year of release	NUtE (kg kg[N]$^{-1}$ha^{-1})
Davis	1965	19.1 B
Santa Rosa	1966	14.1 A
Campos Gerais	1968	21.9 B
IAS3	1973	22.1 B
Paraná Marrom	1974	22.1 B
Paraná	1974	26.1 C
Viçoja	1976	23.0 B
BR4	1979	20.9 B
FT Abyara	1988	25.5 C
FT-11 Alvorada	1985	20.8 B
Ocepar 3 - Primavera	1985	22.0 B
Ocepar 4-Iguaçu	1985	21.7 B
BR16	1985	20.6 B
FT Cometa	1989	21.1 B
BR36	1991	19.6 B
Embrapa 48	1998	22.9 B
MG/BR 46 (Conquista)	1998	21.7 B
CD 206	2003	22.4 B
BRS 245 RR	2005	20.2 B
BRS 282	2007	22.0 B
BRS 284	2009	26.3 C
BMX Potência	2007	25.8 C
AFS 110	2011	27.3 C
Nidera 5909	2012	22.4 B
Cultivar		***
CV%		9

Uppercase letters in columns classify the averages by Scott Knott's test at 5% of significance.

Nitrogen partitioning

Figure 11 illustrates the dynamics of N partitioning in soybean plants at five growth stages. Values are expressed as a percentage (%) in relation to the N accumulated in relation to the sum of N accumulated from the measured plant partitions. In stage R_8, in average, 83% of the total nitrogen accumulation was present in the grains when related to total biomass N.

For stages V4, R1, and R5 there was non-significant ($p > 0.05$) change in N partitioning with cultivar year of release. At R3, N partitioning to the leaf and pod showed a significant ($p < 0.05$) decrease and increase with year of release respectively, while the stem had no change. And at R8, N partitioning to the pod and the grains showed a significant decrease and increase, respectively, while N partitioning to the stem showed no change with year of release ($p < 0.05$) (Figure 11).

The regression made between the NHI:HI ratio and the year of release showed a significant decrease from 1.7 mg g^{-1} 1965 to 1.4 mg g^{-1} 2012 ($p < 0.05$) (Figure 12).

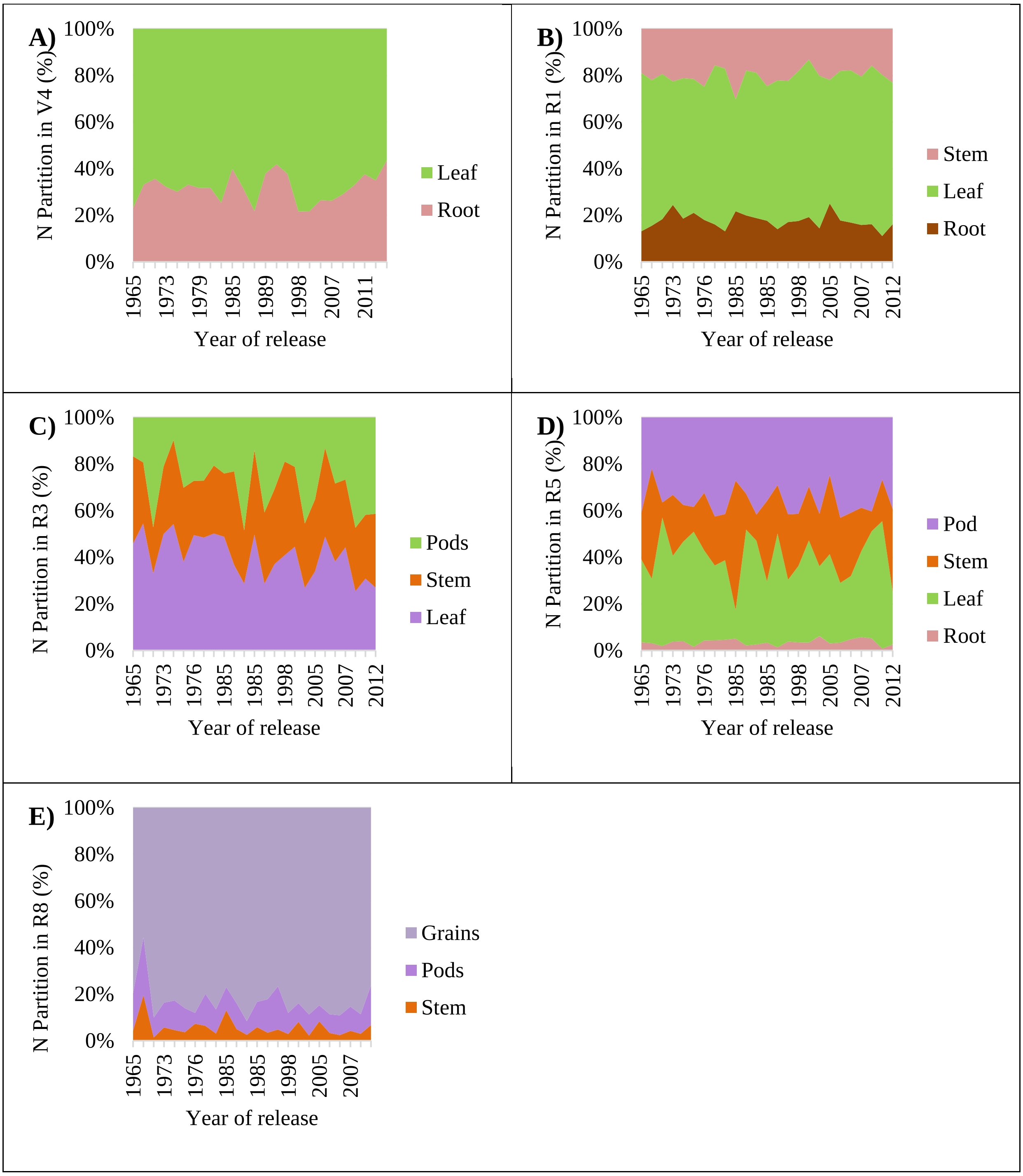

Figure 11. Dynamics of N partition between the plant organs at each growth stage compared with the year of release. A) V_4 B) R_1 C) R_3 D) R_5 E) R_8

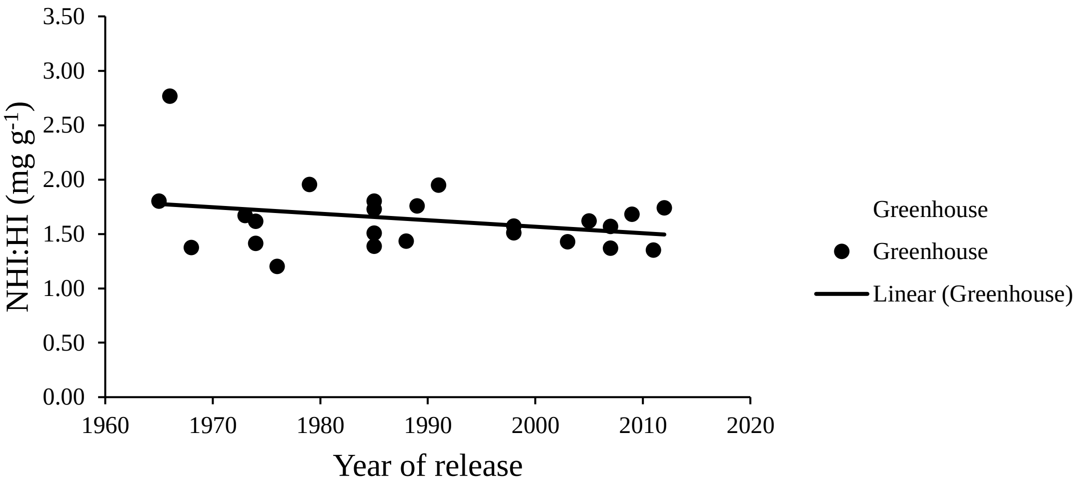

Figure 12. Correlation between the ratio of nitrogen harvest index (NHI) and harvest index (HI) with the year of release for Experiment II. Plotted points are averages in order to facilitate visualization but regressions were performed with all replicates.

5. DISCUSSION

The aim of this study was to evaluate the dynamics of N and its partitioning in the soybean plant in a historical series of soybean cultivars. We found that there was an increase in the total soybean N accumulation with year of release, which was driven by modern cultivars producing more protein-rich grain, as was found by Umburanas (2019). Although the allocation of both dry matter and N to grain increased with year of release, the increase in the N Harvest Index was less pronounced than the increase in the Harvest Index. This seemed to explain the decrease in grain N concentration with year of release. In addition, we observed that modern cultivars produced more grain per quantity of N taken up by the plant (NUtE), which also reflects a dilution of N within the grain. In contrast to this increase in the accumulation of N and dry matter in grains and biomass, the straw presented a decrease in both aspects. With these results, we can more accurately assess the positive and negative outcomes of crop physiological changes and develop insights for new breeding priorities.

Comparing with the literature, the N accumulation and N partitioning presented in our study are quite consistent. The study carried out by Ortez *et al.* (2018) presented a range of N accumulation in the grain in relation to the year of release very close to that found in this study.

The relationships between dry matter (kg ha^{-1}) and N concentration (mg g^{-1}) with year of release are strongly confirmed by several studies found in the literature. Koester *et al.* (2014) carried out a study with cultivars released between the years 1923 to 2007 in the United States and reported that there was an increase in the number of seeds and a higher harvest index (HI). Similar results were found by Suhre *et al.* (2014) with 116 different genotypes and 80 years of release, Kumudini (2001) with two ancient cultivars and two modern cultivars and Todeschini *et al.*, (2019) with 29 cultivars with the years of release from 1965 to 2011. The increase in Harvest Index may be explained by a lower number of branches and shorter plants, which produced less straw but more grain (TODESCHINI *et al.*, 2019).

Regarding the partitioning of N to grains in R8, both experiments showed a significant positive correlation ($p<0.05$) to the year of release. What agrees with the study carried out by Long(2013), in which an increase in N partition in grains in more modern cultivars is also described. This finding suggests that modern soybean cultivars are more efficient than older cultivars at remobilizing N from other plant organs to the grain and/or taking up new N during reproductive growth. However, there is a minimum level of N that must be maintained in the stems and leaves, so further increasing N remobilization may be limited Munier-Jolain(1996) .

Many of the modifications that we observed due to breeding could be considered positive. For example, Umburanas (2019) found that soybean grain yield increased by 3.9 g m^{-2} per year. This is rate of yield increase is greater than what was shown in research by Jin *et al.*, (2011), Long, (2013), Rowntree *et al.*, (2013), who found yield increased around 3.1 g m^{-2} per year in United States and China and around 2.8 g m^{-2} globally. For data from Brazil, an explanation for its higher yield increase rate is the intensive soybean breeding efforts over a relatively short period of time (FISCHER *et al.,* 2014). In particular, the improvement to adapt the crop to warmer biomes such as the Cerrado and northeast regions, which was aided by the long juvenile trait (CONAB, 2021; (FISCHER *et al.*, 2014). The yield increase was due mainly to increased allocation of dry matter to grain. Overall plant biomass did not change. In general the increase in grain yields main objective is to feed the world's growing population (FAO, 2009), either directly or indirectly. However, in addition to the higher yields, modern soybean cultivars are also easier to manage because traits have been integrated into modern germplasm, as glyphosate resistance (MONSANTO, 2021), insect resistance (PINHEIRO, 2006; EMBRAPA, 2018), water stress tolerant (TMG, 2021) and especially in Brazil, temperature tolerance (FPS, 2021). Modern soybean cultivars are able to produce more grain per unit of N uptake than older cultivars. This means that higher yielding modern cultivars do not require proportionally more N and this feature could help to reduce the need for N fertilizer inputs to support high yields, which has been proposed as a management strategy for high yielding systems (La Menza et al. 2017). Also, the efficiency of BNF has gone up (UMBURANAS, 2019; ZAMBON, 2020). Despite greater N utilization efficiency of modern soybean cultivars, N concentration in the straw has remained constant with year of release. The maintenance of straw N concentration could be considered positive in that high straw N concentration favors faster N cycling, which is usually beneficial for the next crop in the rotation (CHAPMAN *et al.*, 2006).

However, the development of cultivars with a clear increase in the efficiency of N use generates cultivars with decreasing content concentration in the grains, which can bring harm to the food use of soybean, since the minimum protein content required in the grain is from 46 to 48% (PÍPOLO and MANDARINO, 2016). Assuming a N-to-protein conversion factor of 6.25, the protein concentration in grain decreased from above the threshold, about 62% until 2000 to below the threshold, about 42% from 2000. It is important to note that the Kjeldahl method (BRADSTREET, 1954) includes a portion of nonprotein N and that the conversion is a general estimate. Our results agree with studies carried out in 2017 La Menza, in which he found that high yielding cultivars, producing around 2500 to 6000 kg ha^{-1} had values between 41 and 38% of protein in the grains. The decreasing trend of soybean protein with year of release is concerning and suggests that future breeding efforts may need to consider both yield and quality.

In addition, the decrease in straw dry matter and N accumulation found in this study could contribute to a partial negative balance of N in the soil, resulting in depletion of soil N stocks over time, corroborating the assertion of the authors of the area (SALVAGIOTTI *et al.*, 2008; CIAMPITTI and SALVAGIOTTI, 2018; ZAMBON, 2020). When Blumenthal *et al*, (1988) evaluated the N return by soybean straw, they found that increasing plant population did not lead to greater residual N return and no benefit for the partial N balance in the soil. Our results imply that alternative treatments for N replacement in the soil may be needed in soybean cropping systems, such as rotation with cover species or green manure.

For future studies on this topic, it is suggested to further explore the analyses aimed at the protein concentration and composition in grains and check if it is still within the limits approved by the market. We suggest further study on the allocation of N at different growth stages during soybean development, because interpretation of the greenhouse results was limited by the loss of some samples.

6. CONCLUNSION

In this study, we determined the N dynamics and partitioning for a historical series of soybean cultivars from Brazil. We found that the breeding programs increased the N content in the grain, but the dry matter in the grain had a more intense increase, generating a lower N concentration in the grain. The decline in grain N concentration may compromise the quality of the product. Thus, it is important to consider both grain yield and grain protein in future breeding efforts. Lastly, declining straw dry matter and N returns to the soil implies that management practices may need to be implemented to sustain soil N stocks in soybean fields.

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