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Dynamics of Soybean Seed Protein and Oil Content with Depth in the Canopy

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by

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Dynamics of Soybean Seed Protein and Oil Content with Depth in the Canopy

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Understanding the physiological basis of variation in seed composition is critical for optimizing soybean seed composition. Also, studying dry matter accumulation and nitrogen uptake, partitioning, and redistribution in soybeans is crucial due to its direct impact on seed yield levels and overall plant productivity. This study aimed to test the primary proxy influencing seed protein and oil content, which is their accumulation rate determined by the assimilate supply per seed. The second objective was to comprehensively understand soybean dry matter (DM) accumulation and nitrogen (N) uptake, partitioning, and removal patterns within the canopy. Field experiments were conducted using two soybean varieties, Hoegemeyer LL2841 (high-protein concentration) and Pioneer P27A17X (low-protein concentration), planted at early, mid, and late dates. The first study showed that at the whole plant level, protein and oil accumulation rates accounted for over 51% of the variation in seed component contents, with leaf area per seed at the R5.5 stage explaining more than 66%. However, within individual canopy strata, the relationship between assimilate supply and accumulation rates was less consistent. Protein accumulation rates significantly influenced protein content in strata 1, 3, and 4, while oil accumulation rates were significant only in strata 4 and 5. The second study indicated that whole plant accumulation of DM and N is

minimal until approximately R1, then increases rapidly to the peak near R3 for all treatments. After R5, vegetative organs (leaves and petioles) generally began remobilizing DM and N to seed, whereas stems began DM and N remobilization at R6, possibly supporting greater DM remobilization to the seed. Within strata, peak accumulation of DM and N shifted from R1 to R7 from the bottom of the plant (strata 1) to the top (strata 5). Moreover, vegetative organs varied in their time of maximum DM or N accumulation, and when remobilization of DM or N to seed was initiated. Our results showed that at the whole plant level, seed N accumulation primarily relied on remobilization from vegetative organs rather than on continued uptake. Strata 2 and 3 contributed most of remobilized N to seed.

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Chapter 1

Linking Assimilate Supply to Soybean Seed Composition with Canopy Depth

Introduction

Meeting the minimum protein concentration values is crucial to ensure cost-effective soybean meal marketing (Bednar et al., 2000; Kristófersson & Anderson, 2006; Adrangi et al., 2011; Moran et al., 2017; Bumhira & Madzimure, 2023). Intrinsic and extrinsic factors play a significant role in influencing protein and oil concentration in soybeans. Intrinsic factors such as genetic variation, seed size, mineral composition, and plant physiology have been shown to impact the protein content of soybean seeds (Wilson et al., 1995; Gibson & Mullen, 1996; Bethlenfalvay et al., 1997; Rotundo et al., 2009; Montanha et al., 2022). Extrinsic factors such as temperature, nitrogen application, latitude, and other environmental factors have also been demonstrated to influence soybean seed protein concentration (Wilson et al.,1995; Goldflus et al., 2006; Rotundo & Westgate, 2009; Assefa et al., 2019). Understanding the physiological basis of variation in seed composition is critical for optimizing soybean seed composition, and the need for cost-effective soybean meal marketing underscores the urgency of this understanding.

The key factors driving soybean seed protein and oil content are the rate and duration of seed component accumulation, which are intricately linked to assimilate supply (Rotundo et al. 2009, 2011). Numerous field studies have attempted to associate seed composition with assimilate supply during seed fill, but with limited success (Rotundo et al., 2009 and citations therein). Rotundo et al. (2009) argued that assessing response in seed composition to assimilate supply per seed is more robust than using assimilate supply per plant. The standard proxy for measuring assimilate supply per seed is leaf area (cm^2 $seed^{-1}$) or leaf nitrogen (mg $seed^{-1}$) at the start of the linear phase of protein and oil accumulation (~R5) because leaves are the primary source of carbon and nitrogen accumulated during grain fill (Rotundo et al., 2009). Rotundo et al. (2011) found that seed protein or oil accumulation rate was more important than the duration of accumulation in driving final seed protein or oil content. Assimilate supply per seed is determined by both intrinsic and extrinsic factors. Rotundo et al. (2011) showed variation in assimilate supply per seed among genotypes and manipulations in assimilate supply (de-podding or shading). They were able to explain the relationship between assimilate supply per seed and the rate of component accumulation using a single function across genotypes and manipulations in assimilate supply.

The protein concentration in soybean seeds exhibits variability depending on their node position on the plant. Moro Rosso et al. (2021) demonstrated that soybean seeds from upper nodes exhibited higher protein and lower oil concentrations, attributing these differences to the vertical canopy profile and the impact of branches on soybean seed composition. Protein concentration increased linearly from the lowest to the highest node in determinate soybeans, with similar trends observed in indeterminate types (Bellaloui & Gillen, 2010). Conversely, lower nodes on the plant tend to have greater oil concentration than upper nodes (Escalante & Wilcox, 1993). During seed fill, 30% of the carbon assimilated in leaves is remobilized to pods on the same node and another 30-40% to nearby nodes (Stephenson & Wilson, 1977; Moro Roso et al., 2021). Owing to differences in incident radiation, air temperature, carbon dioxide concentration at the leaf surface, and vapor pressure deficit (Moro Roso et al., 2021), assimilate supply per seed is likely to vary considerably with canopy depth, which may contribute to differences in seed composition within the canopy.

Planting date has been shown to play an essential role in determining soybean seed composition. An early planting date results in greater yields than later planting dates because plants have an extended vegetative period, allowing for greater vegetative biomass accumulation (Bellaloui et al., 2015) and potentially large variation in assimilate supply per seed. However, early planting also lowers protein concentration (Dardanelli et al., 2006; Mourtzinis et al., 2017; Assefa et al., 2019). Further research is needed to explore the underlying mechanisms and identify the factors influencing protein and oil accumulation at different node positions within the soybean canopy.

We hypothesize that the protein and oil accumulation rate primarily influence seed protein and oil content. Furthermore, this rate is primarily determined by the assimilate supply per seed, regardless of the seed's position in the canopy profile. To test these hypotheses, we cultivated two soybean varieties with different protein concentration at three planting dates to create varying maturation environments.

Materials and Methods

The site and plant culture

A field study was conducted at an experimental farm on the University of Nebraska-Lincoln campus in 2020 and 2021. The soil type at the farm is a Kennebec silt loam (fine-silty, mixed, super active, mesic Cumulic Hapludoll). The varieties selected for this study were known to differ in total protein concentration based on preliminary experiments used to measure the protein concentration of 73 soybean varieties in farmers' fields in Nebraska (Carciochi et al., 2023). Pioneer P27A17X (MG 2.7) was selected for its lower seed protein concentration (33.5%) and Hoegemeyer LL2841 (MG 2.8) was selected for its greater protein concentration (35.7%). Three planting dates were selected to be approximately 200 growing degree days (GDD) apart based on 20 years of weather data in Nebraska, about two weeks apart. Sowing occurred on April 27, May 18, and June 8, 2020, and on April 26, May 18, and June 3, 2021.

Soybeans were planted using a six-row planter with a 0.76-m row spacing. The target seeding rate was 432,250 seeds per hectare to minimize soybean branching. Weeds were manually controlled during the growing season until canopy closure. No insect or pathogen management was implemented in this study. Each experimental unit consisted of 20 rows of soybeans, which spanned 15.2 meters by 12.8 meters in length.

Sampling methods

Each experimental unit had multiple subplots designated for destructive sampling. The sampling subplots were started from the third row of the plot, leaving an undisturbed row between sample areas to prevent edge effects. At least 1 m was left undisturbed to avoid edge effects within sampled rows. The four middle rows of each experimental unit were left untouched to allow harvesting of the final stand yield from the central two rows. A one-meter-long plastic net was placed in between rows within the center four rows of each experimental unit beginning at R1 to collect fallen leaves and petioles weekly to determine abscised leaf and petiole mass and N content. Final yield was measured using a combine harvester in the two central rows, leaving at least 2 meters at the plot edge. Yield is reported on a 13% moisture content basis.

Plants in each 1 m subplot were clipped at the cotyledon node and brought to the lab for processing each week beginning one week after emergence. Number of plants per subsample were counted and ten plants randomly selected to assess growth stage.. All plants within subsample were then divided into five canopy layers, herein referred to as strata, based on the number of nodes. The counting of the strata was done from the soil surface upwards, with the first strata including nodes 1-5, the second strata including nodes 6-9, the third strata including nodes 10-13, the fourth strata including nodes 14-17, and the fifth strata including node 18 to the top of the plant. Within each strata, plants were separated by their green leaf lamina, petiole, stem, pod, senesced leaf, and fallen leaf. The green leaf area within each strata was determined by processing the green leaves using a LICOR-3100 area meter. Organ groups were then bagged and placed in the dryer at 60 C to constant mass. Dried tissue samples were then ground to a size of 1 mm using a Wiley Mill (Thomas Wiley Mills model 4). From seed fill (R5) until maturity (R8), after drying, reproductive organs were separated into seeds and pod walls. Seeds were also ground using a coffee grinder (BODUM Electric) to avoid contamination and oil loss.

Accumulation of seed components and assimilate supply per seed

Seed component concentration and content data are presented as a percentage of final seed weight (%) at 13% moisture content, and component content is expressed on a mass-per-seed basis (mg seed^{-1}). Nitrogen content was analyzed from seed fill (R5) to maturity (R8). The subsamples' seed nitrogen (N) concentration was determined by combustion using Leco FP-528 on 0.25-0.26 g dry ground samples. Seed protein content was estimated as percent N concentration x 6.25 x seed mass on dry matter basis and then converted to 13% moisture content (FAO, 2002). Oil concentration was determined using the accelerated solvent extraction (ASE) method (Matthaus & Bruhl, 2001) with modifications to the hexane evaporation process using the Genevac Rocket Synergy Evaporation System. Seed oil content was calculated by percent oil concentration x seed mass in dry basis and then converted to 13% moisture content. Residual content was determined by the difference in total seed mass by protein and oil content.

The rate of protein, oil, and residual accumulation was estimated using a bi-linear model with plateau using GraphPad Prism (GraphPad Software, San Diego, CA, USA), with the following equation (Rotundo, 2011):

Component content (mg seed^{-1}) = $a + b * TT$ for $TT < c$

Component content (mg seed^{-1}) = $a + bc$ for $TT > c$

where TT is thermal time after soybean stage R5.5 in degree days (ºCd with a base temperature of 7.6 C), a is the y-intercept (mg seed^{-1}), b is the rate of component accumulation during the linear phase of seed filling (mg seed^{-1} ºCd^{-1}), and c is the crossing point between a linear phase of component accumulation and the maximum seed component content defined by the plateau function.

Assimilate supply per seed within each strata was estimated as the ratio between green leaf area at the beginning of seed fill (R5.5) and total seed number within that strata at maturity. If a destructive sample did not occur on the day R5.5 was reached, the leaf area within a strata was estimated by extrapolation between sampling dates.

Experimental design and statistical analysis

The experiment was designed using a completely randomized design and a split-plot factorial with four replications for 2020 and 2021, respectively. The main plot consisted of planting dates, while soybean variety was used as subplots. In the first year, all replications were randomized within planting date with the assumption that the confounder effect was not present, and in the second year, all treatments were randomized in replicate blocks.

Statistical analyses were performed to compare the mean whole plant seed component concentration and content among treatments using the mixed procedure in SAS (SAS Institute, Cary, NC). Least squares means were compared using the Tukey grouping test at the 0.05 probability level. The statistical model included variety and planting date as fixed effects in 2020 and 2021 and planting date by replication as random effects in 2021.

The rate of seed component accumulation was regressed on assimilate supply (leaf area per seed within strata) following the Michaelis-Menton kinetics approach outlined in Rotundo et al. (2011). Parameters were estimated using GraphPad Prism, and confidence intervals were used to estimate significant differences among seed component parameters.

Results

Environmental conditions

Across all environments, growing conditions were mostly favorable, with slight temperature deviations from the 30-year average. The average temperatures during the growing season from May to September were 21.8 and 22.5 C for 2020 and 2021, respectively. In 2020, May, August, and September temperatures were slightly lower than the 30-year average, whereas June and July were slightly greater (Table 1.1). Average temperatures were equivalent to or greater than the 30-year average throughout the growing season in 2021.

Table 1.1 Monthly average temperature and cumulative precipitation during the growing season and average 30-year data.

Month	Temperature (^{o} C)			Precipitation (mm)		
	2020	2021	Average 30 years	2020	2021	Average 30 years
May	15.5	16.5	16.4	83	67	132
June	25.3	24.6	22.2	90	88	121
July	25.5	24.7	24.6	131	56	95
August	24.1	25.3	23.3	31	86	91
Sept	18.5	21.3	18.9	61	13	76

Data was collected from the High Plains Regional Climate Center.

May and June precipitation was slightly greater in 2020 but much lower than the 30-year average in both years (Table 1.1). July precipitation was 38% greater than the 30-year average in 2020 but 41% lower in 2021. August precipitation was 66% lower than the 30-year average in 2020 but about equal in 2021. September precipitation was near the 30-year average in 2020 but 83% lower in 2021. Soil fertility in the fields was adequate for soybean production (Table 1.2).

Table 1.2 Soil fertility characteristics during 2020 and 2021.

Year	pH	OM†	Nitrate	P	K	Ca	Mg	Na	S
		g kg^{-1}	-------------------------------mg kg^{-1}-------------------------------						
2020	6.1	25	7.1	68.6	238.3	1658.4	241.4	14.1	9.0
2021	6.5	47	4.5	38.0	306.0	2211.0	256.0	13.0	7.5

† Organic matter (OM)

Whole plant yield, protein, and oil concentration

The experiment was conducted over two years using different research designs. Since the statistical model differed for each year, we present the data separately by year. There was a planting date by variety interaction for soybean yield at R8 in both years (Table 1.3). The yield of Hoegemeyer LL2841 generally declined with later planting dates in 2020, whereas Pioneer P27A17X did not vary among planting dates (Figure 1.1). However, Hoegemeyer LL2841 had the greatest yield in the mid-planting date in 2021, whereas the yield of Pioneer P27A17X again did not vary among planting dates.

There was no planting date by variety interaction for seed protein concentration at the R8 stage in either year (Table 1.3). Grain protein concentration differed among varieties in both years but only among planting dates in 2021. Opposite of our expectation, Pioneer P27A17X protein concentration was 1.0 and 1.8% greater than Hoegemeyer LL2841 in 2020 and 2021, respectively (Table 1.4). Pioneer P27A17X seed protein concentration was reduced by 3.8% in the late planting date treatment in 2021.

A planting date by variety interaction for seed oil concentration occurred in 2021, whereas soybean seed oil concentration only varied among varieties in 2020. (Table 1.3). Hoegemeyer LL2841 had a greater (20.06 ± 0.20%) seed oil concentration compared to Pioneer P27A17X (19.82 ± 0.19%) in 2020. Hoegemeyer LL2841 seed oil concentration did not vary with the planting date in 2021, whereas Pioneer P27A17X was only greater in the late planting date (Figure 1.2).

Table 1.3 Analysis of variance of soybean seed yield, protein, and oil concentration at R8 and protein, oil, and residual content (mg seed^{-1}) at R7.

Source	DF	Pr > F					
		Yield	Protein % R8	Oil % R8	Protein content R7	Oil content R7	Residual content R7
		---Year 2020---					
Planting Date	2	0.010	0.19	0.74	<.0001	<.0001	<.0001
Variety	1	0.440	0.02	0.009	<.0001	<.0001	<.0001
Planting Date*Variety	2	0.003	0.07	0.26	0.0014	0.0012	0.011
Residual	18						
		---Year 2021---					
Planting Date	2	0.004	0.0003	0.0005	<.0001	0.0002	0.012
Variety	1	0.002	0.017	0.93	<.0001	<.0001	<.0001
Planting Date*Variety	2	0.045	0.461	0.03	0.25	0.029	0.032
Planting Date*Rep	9	0.480	0.688	0.63	0.16	0.296	0.24
Residual	9						

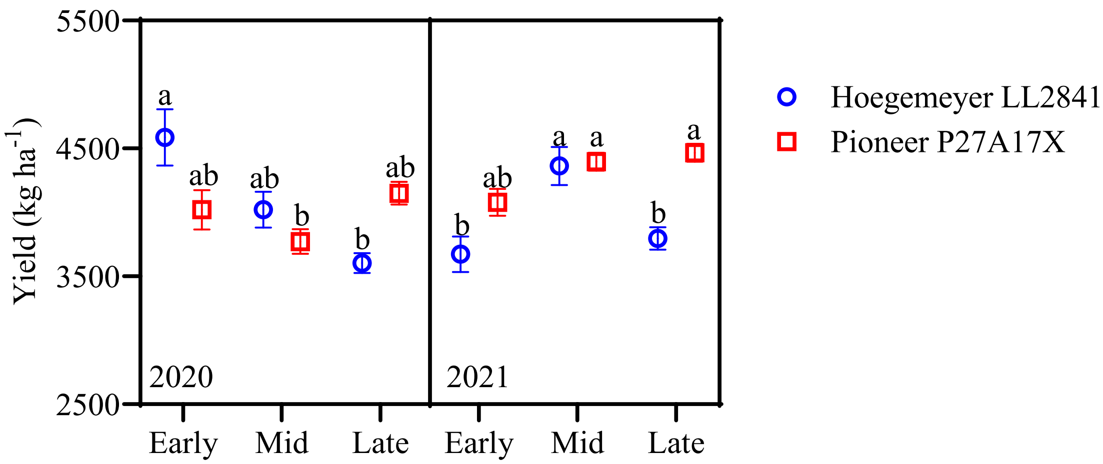

Figure 1.1 Soybean yield of two varieties at three different planting dates in 2020 and 2021. Tukey grouping for LS-means (Alpha=0.05) indicated with the same letter in the same year is not significantly different.

Table 1.4 Soybean seed protein concentration of two different varieties in 2020 and 2021 at R8 stage.

Variety	Protein % in 2020*	Protein % in 2021*
Hoegemeyer LL2841	34.18 ± 0.11 b	33.06 ± 0.13 b
Pioneer P27A17X	34.54 ± 0.11 a	33.64 ± 0.13 a

* Tukey grouping for LS-means (Alpha=0.05). LS-means indicated with the same letter in the same column are not significantly different. LS error was added after the mean value.

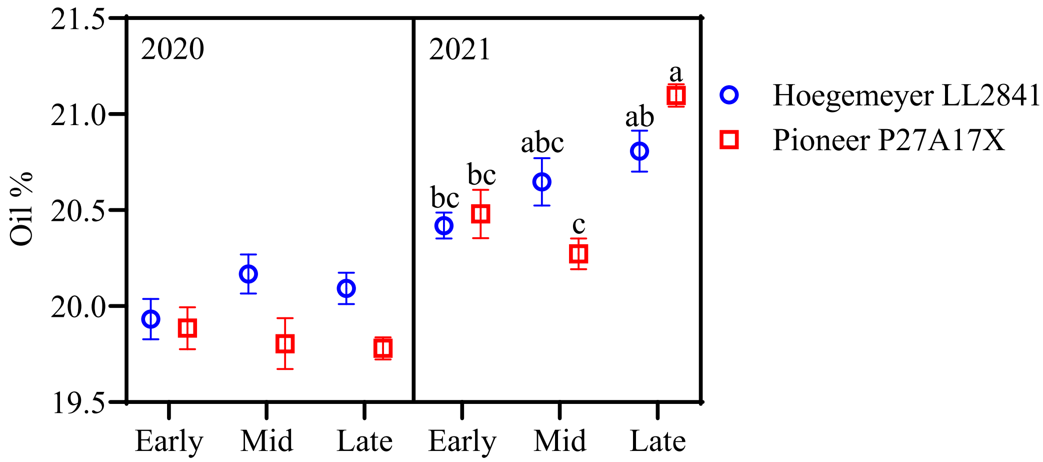

Figure 1.2 Soybean seed oil concentration in two varieties and three different planting dates in 2021 at the R8 stage. The Tukey grouping for LS-means (Alpha=0.05) indicated with the same letter is not significantly different.

Whole plant protein, oil, and residual content at the R7 stage of development

Soybean seed protein and oil content are determined from seed protein and oil concentration and seed mass. A planting date by variety interaction for soybean seed protein content (mg seed^{-1}) occurred in 2020 but not in 2021 (Table 1.3). Hoegemeyer LL2841 had greater seed protein content than Pioneer P27A17X in 2020, and protein content increased with later planting dates for both varieties, but the trend was not as strong for Hoegemeyer LL2841 (Figure 1.3). Hoegemeyer LL2841 also had greater seed protein content compared to Pioneer P27A17X, and both varieties increased protein content with later planting dates in 2021.

A planting date by variety interaction for soybean seed oil and residual content (mg seed^{-1}) was observed in both years of the study (Table 1.3). Hoegemeyer LL2841 had greater seed oil content than Pioneer P27A17X in both years. Both varieties increased seed oil content with later planting dates, but the trend was less strong in 2021 than in 2020 (Figure 1.4). Similarly, Hoegemeyer LL2841 had greater residual content than Pioneer P27A17X, and residual content increased with later planting dates for both varieties in 2020. However, while Hoegemeyer LL2841 had greater residual content in 2021, there was a slight variation in residual content among planting dates in that year (Figure 1.5).

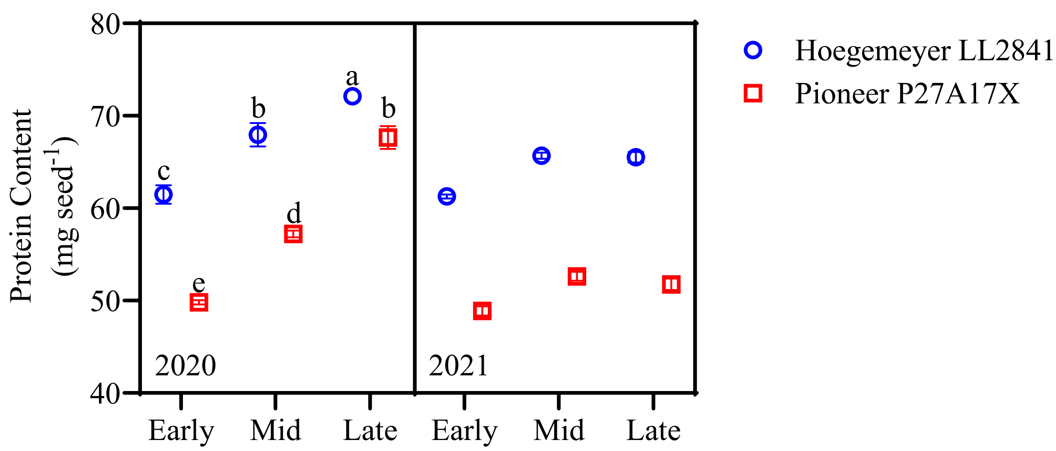

Figure 1.3 Soybean seed protein content (mg seed^{-1}) in two varieties and three different planting dates in 2020 and 2021 at the R7 stage. The Tukey grouping for LS-means (Alpha=0.05) indicated with the same letter is not significantly different.

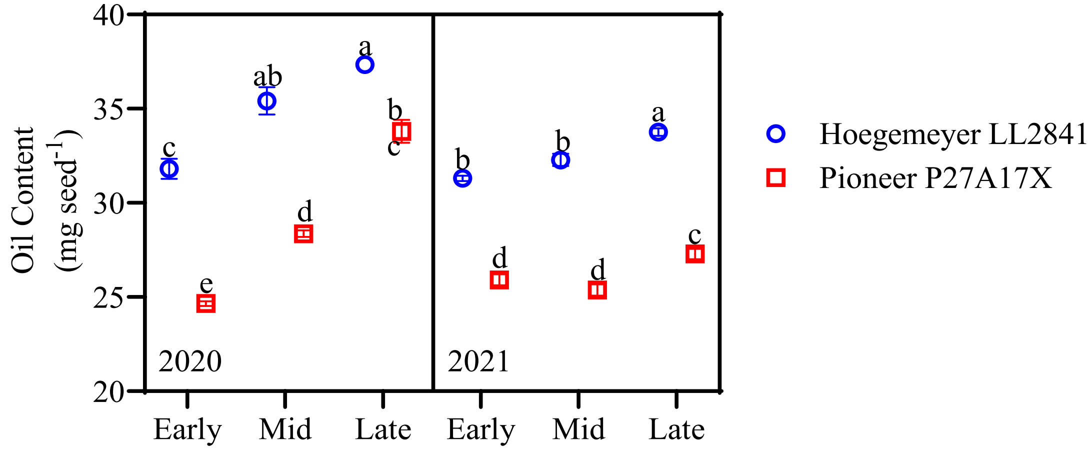

Figure 1.4 Soybean seed oil content (mg seed^{-1}) in two varieties and three different planting dates in 2020 and 2021 at the R7 stage. The Tukey grouping for LS-means (Alpha=0.05) indicated with the same letter in the same year is not significantly different.

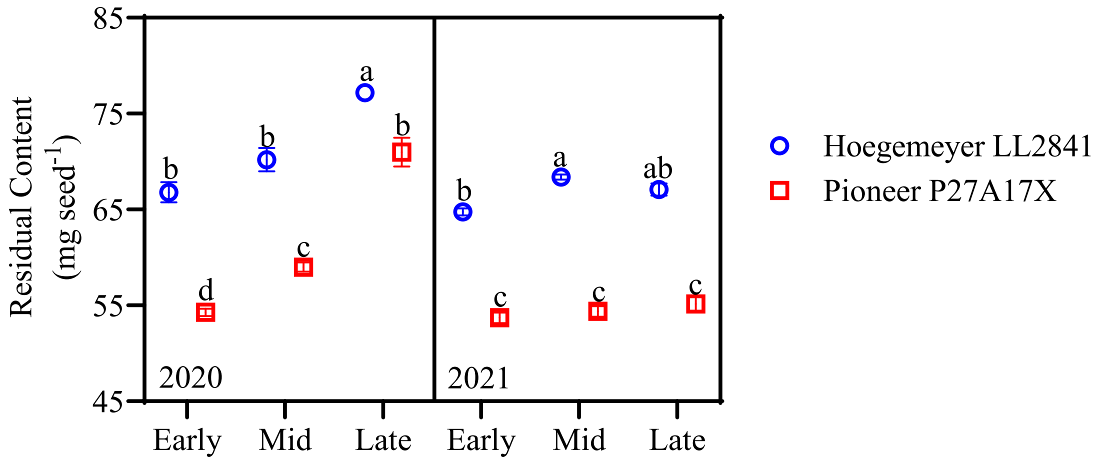

Figure 1.5 Soybean seed residual content (mg seed^{-1}) in two varieties and three different planting dates in 2020 and 2021 at the R7 stage. Tukey grouping for LS-means (Alpha=0.05) indicated with the same letter in the same year is not significantly different.

Protein and oil content depends on their rate of accumulation

Variation in seed component content at R7 may depend on the rate and duration of component accumulation. The protein and oil accumulation rate explained more than 51% of the variation in seed component content across planting dates and varieties at the whole plant level (Figure 1.6). The rate of content accumulation did not differ among planting date or variety treatments. In our study, the duration of component accumulation did not explain any differences in seed component content at the whole plant level, with P values of 0.68 and 0.36 for protein and oil content, respectively.

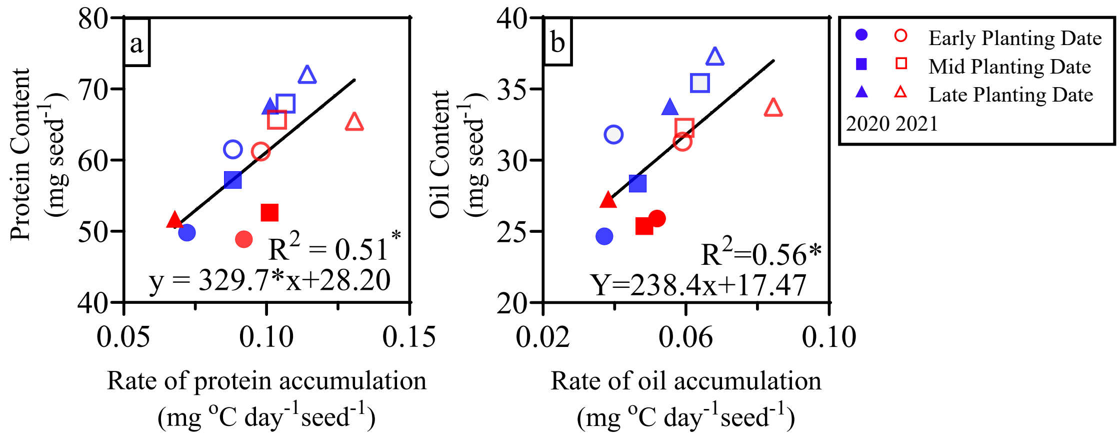

Figure 1.6 Relationship between seed component content and the rate of component accumulation at the whole plant level for three different planting dates and two varieties. Open symbols: Hoegemeyer LL2841; closed symbols: Pioneer P27A17X; blue color: the year 2020; red color: the year 2021; circle: early planting date; square: mid planting date; and triangle: late planting date. Each point is the average of four replications.

The relationship between component content and the rate of component accumulation was not as consistent across strata within the canopy. The rate of protein accumulation within strata explained a significant amount of variation in seed protein content within that stratum for strata 1, 3, and 4 but not for strata 2 and 5 (Figure 1.7a). On the other hand, the rate of oil accumulation within strata only explained a significant amount of variation in seed oil content within that stratum for strata 4 and 5 (Figure 1.7b). Across all strata, the duration of protein accumulation within those strata did not explain component content within those strata (Figure 1.8a). The protein accumulation duration had P values of 0.65, 0.61, 0.50, 0.70, and 0.75 for strata 1, 2, 3, 4, and 5, respectively. Furthermore, the duration of oil accumulation only explained the variation in seed oil content in strata 5 (Figure 1.8b). The oil accumulation duration had P values of 0.84, 0.67, 0.96, 0.26, and 0.05 for strata 1, 2, 3, 4, and 5, respectively.

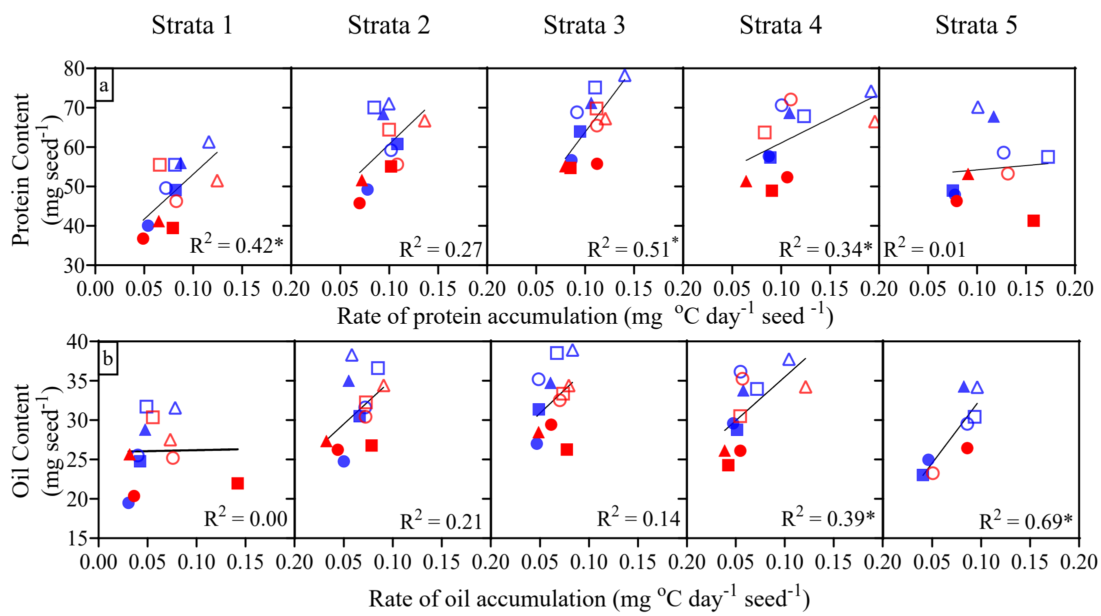

Figure 1.7 Relationship between seed component content within a strata and the rate of component accumulation in that strata for three planting dates and two varieties. Open symbols: Hoegemeyer LL2841; closed symbols: Pioneer P27A17X; blue color: the year 2020; red color: the year 2021; circle: early planting date; square: mid planting date; and triangle: late planting date. Each point is the average of four replications. An asterisk indicates that the regression explained a significant proportion of component content at P<0.05.

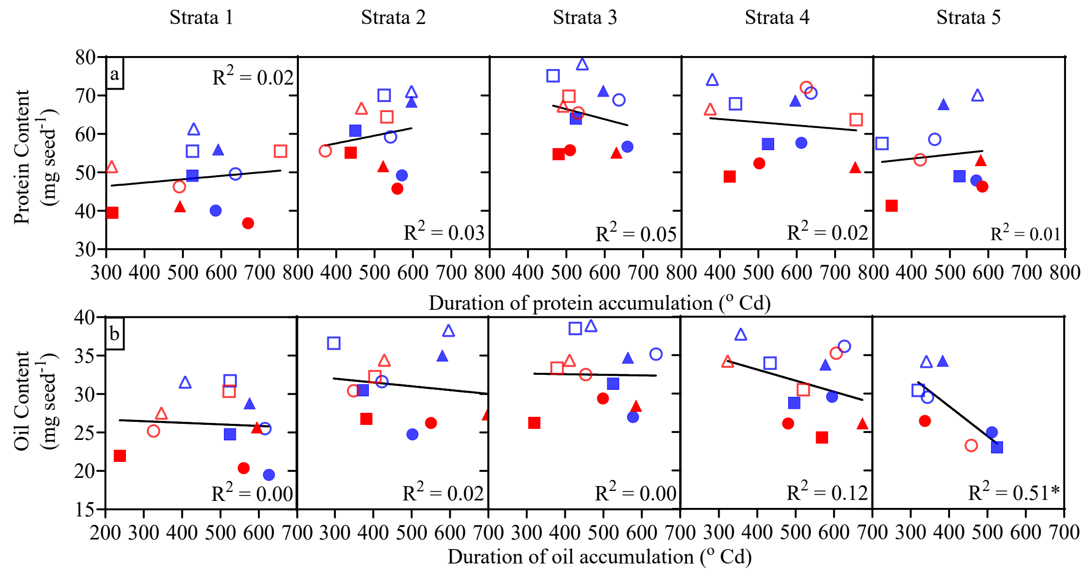

Figure 1.8 Relationship between seed component content within a strata and the duration of component accumulation in that strata for three planting dates and two varieties. Open symbols: Hoegemeyer LL2841; closed symbols: Pioneer P27A17X; blue color: the year 2020; red color: the year 2021; circle: early planting date; square: mid planting date; and triangle: late planting date. Each point is the average of four replications. An asterisk indicates that the regression explained a significant proportion of component content at $P<0.05$.

Do protein and oil accumulation rates depend upon assimilate supply per seed?

It has been argued that the component accumulation rate depends upon the amount of available assimilate supply (Rotundo et al., 2011). One proxy to measure available assimilate supply to the source is leaf area per seed at R5.5. We examined the relationship between the rate of component accumulation and leaf area per seed both at the whole plant level and within each strata. Leaf area per seed explained more than 66% of the variation in the rate of component accumulation at the whole plant level (Figure 1.9).

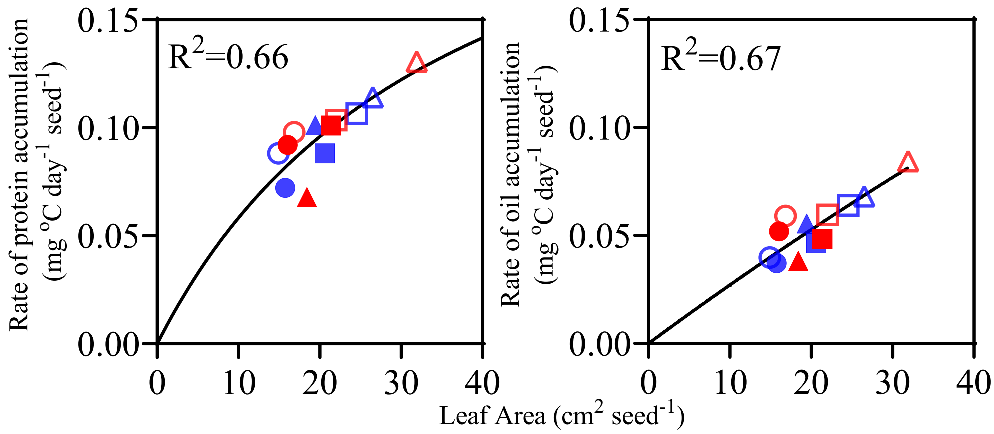

Figure 1.9 Relationships between protein and oil accumulation rate and assimilate supply per seed (leaf area per seed at R5.5) at the whole plant level for three different planting dates and two varieties. Open symbols: Hoegemeyer LL2841; closed symbols: Pioneer P27A17X; blue color: the year 2020; red color: the year 2021; circle: early planting date; square: mid planting date; and triangle: late planting date. Each point is the average of four replications.

However, when exploring the rate of component accumulation within a strata in relation to the leaf area per seed within that strata, the latter did not consistently explain the variation in the rate of component accumulation (Figure 1.10). Leaf area per seed at R5.5 explained more than 22% of the variation in the rate of protein accumulation in strata 3 and 4 (Figure 1.10a). The same trend also showed that leaf area per seed at R5.5 contributes to the variation of oil accumulation rate in strata 3 and 4 (Figure 1.10b). This proxy (leaf area per seed at R5.5) did not explain variation in protein and oil accumulation rates in other plant strata.

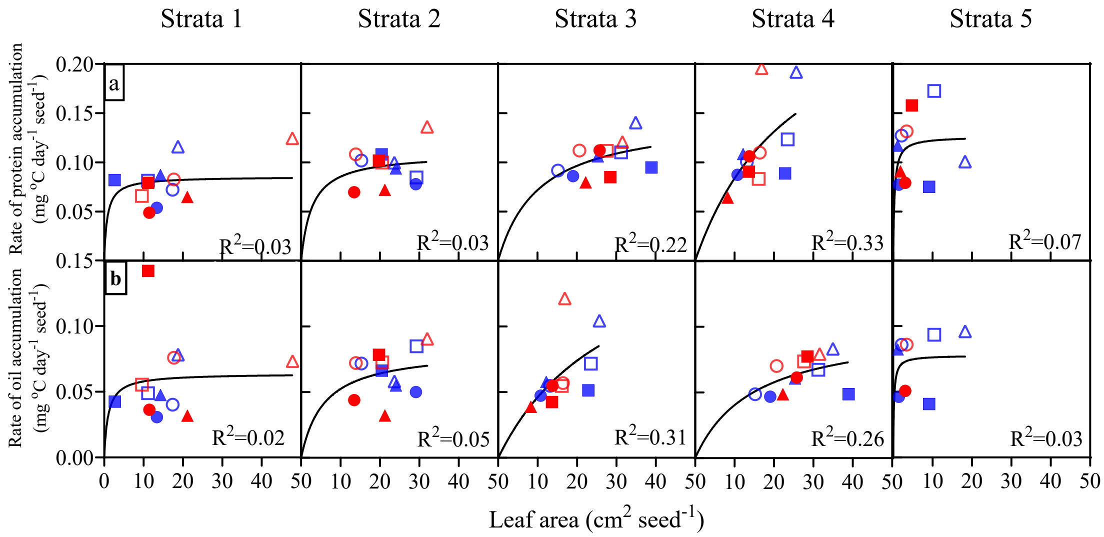

Figure 1.10 Relationships between rate of (a) protein and (b) oil accumulation and assimilate supply per seed (leaf area per seed at R5.5) within each strata for three planting dates and two varieties. Open symbols: Hoegemeyer LL2841; closed symbols: Pioneer P27A17X; blue color: the year 2020; red color: the year 2021; circle: early planting date; square: mid planting date; and triangle: late planting date. Each point is the average of four replications.

Discussion

The two varieties used in this study showed different responses to planting date treatments. In 2020, Hoegemeyer LL2841 yielded the most when planted early and yield declined with later planting dates (Figure 1.1). However, in 2021, Hoegemeyer LL2841 had the greatest yield at the mid-planting date and a similar yield at the early and late planting dates. In contrast, Pioneer P27A17X yield did not vary among planting dates in either year. Existing literature consistently supports that planting date affects soybean yield, with early planting generally maximizing yield (Parker et al., 1981; Li et al., 2014; Knott et al., 2019; Morris et al., 2021). However, specific trends can vary based on environmental conditions, variety maturity, and other factors, highlighting the complexity of this relationship (Grichar et al., 2008; Nleya et al., 2020; France et al., 2022).

Salmerón et al. (2022) found that early planting dates can lead to a dilution or concentrating effect on seed protein and oil concentration, respectively. Similarly, Rowntree et al. (2013) and Bellaloui et al. (2011) reported that delayed planting increased seed protein concentration and decreased seed oil concentration. In contrast, Pott et al. (2019) observed that early planting dates resulted in the greatest protein and oil concentrations, while late planting dates presented the lowest concentrations for these seed quality components. In our study, Pioneer P27A17X had greater seed protein concentration than Hoegemeyer LL2841 in both years of this study (Figure 1.3). The seed protein concentration of Hoegemeyer LL2841 was lower in the late planting date treatment in 2021, but otherwise, neither variety was influenced by planting date in both years. Hoegemeyer LL2841 seed oil concentration was greater than Pioneer P27A17X in both years but did not vary among planting date treatments in 2020 (Figure 1.4). Hoegemeyer LL2841 seed oil concentration did not vary among planting date treatments in 2021, whereas Pioneer P27A17X seed oil concentration was greatest in the late planting date treatment.

Soybean seed protein and oil content are determined from their concentration and seed mass. Therefore, factors that influence seed mass have a large impact on seed protein and oil content. In general, seed protein and oil content increased with later planting dates at the whole plant level, as shown in Figure 1.3 and 1.4. This finding was unexpected, as it contradicted the commonly observed negative correlation between seed oil and protein content (Stombaugh et al., 2003; Matsuo et al., 2016; La et al., 2019; Goettel et al., 2022). Other studies have also reported conflicting results, indicating that delayed planting can decrease protein content and increase oil content (Sugimoto et al., 1998; Phippen & Phippen, 2012; Tokura et al., 2021). These varied findings suggest that the relationship between planting date and soybean seed composition is intricate and may be influenced by temperature, genotype, and environmental conditions.

The impact of planting date on seed composition is significant, with the environment (location and planting date) explaining a large portion of the variation in soybean seed oil and protein concentration (Salmerón et al., 2022). The timing of planting affects the growth and development patterns of soybean seed, consequently influencing the content of protein, oil, carbohydrates, and other chemical components in the seed (Li et al., 2014). This influence is rooted in the relationship between the planting date and the conditions during the seed-filling period. For example, cooler temperatures during the seed-filling period, more common with later planting dates, can lead to changes in seed composition, impacting protein and oil concentrations (Jaureguy et al., 2013). Furthermore, moisture stress at different pod fill stages can also influence seed composition, with higher protein and lower oil concentrations associated with late moisture stress and vice versa (Beckendorf et al., 2008).

The results indicate that the rate of seed component accumulation was the main factor influencing soybean seed component content at the whole plant level (Figure 1.6). Interestingly, the seed-filling duration did not significantly impact component content. This finding is consistent with Rotundo et al. (2011), who suggested that the component accumulation rate accounted for over 68% of the variation in protein, oil, and residual content. However, while the component accumulation rate within a strata partially explained the component content variation in some strata, it did not explain the variation in all strata. For instance, the relationship between seed protein content and protein accumulation rate was significant in the lowest and middle strata, while the oil content-oil accumulation rate relationship was only significant in the two upper strata. This suggests that the component accumulation rate may not be the sole determinant of seed component content in all layers in the canopy. It is possible that certain molecular mechanisms play a critical role in determining seed component content. Du et al. (2020) suggested that sucrose metabolism, transport, and allocation influence seed composition during different seed development stages, particularly under drought stress conditions. They also highlighted that the regulation of metabolic pathways, including carbon fixation, photosynthesis, and fatty acid biosynthesis, is associated with the dynamic changes in oil accumulation during seed development in soybeans (Yang et al., 2019; Xu et al., 2022).

The protein and oil accumulation rates seem to be mainly determined by assimilate supply at the whole plant level, with more than 66% of the variation in these rates being attributable to this factor. Rotundo et al. (2011) suggested that the content of seed components is linked to assimilate supply, using the hyperbolic model proposed by Jenner et al. (1991), and noted that the response varies for each seed component. Meckel et al. (1984) suggested that the rate of dry matter accumulation in soybean seeds is relatively insensitive to fluctuations in photosynthetic rate and assimilate supply. However, Chiluwal et al. (2022) conducted experiments to measure the effect of assimilate supply during the seed-filling phase on the final seed weight of soybeans, revealing a direct relationship between assimilate supply and seed weight. However, this relationship may not be evident at the plant strata level. The leaf area at R5.5, used as a proxy to gauge assimilate supply, may not demonstrate its effect on the rate of component accumulation due to other influencing factors. Additionally, within a given strata, the time at which seed fill begins may differ from the whole plant R5.5 stage, meaning that the leaf area at R5.5 may not represent the actual assimilate supply within that strata.

Conclusion

We conclude that the rate of protein and oil accumulation was a critical factor influencing their final content in soybean seeds, significantly dependent on the assimilate supply per seed. At the whole plant level, accumulation rates explained over 51% of the variation in seed component contents, with leaf area per seed at R5.5 accounting for more than 66% of this variation. This highlights the crucial role of assimilate availability in determining accumulation rates. However, within individual canopy strata, the relationship between assimilate supply and accumulation rate was less consistent, with protein accumulation rates significantly influencing content in strata 1, 3, and 4, and oil accumulation rates in strata 4 and 5. This suggests that, while assimilate supply is a major determinant at the whole plant level, localized factors and molecular mechanisms may play important roles within different plant strata.

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Chapter 2

Dry Matter Accumulation and Nitrogen Uptake, Partitioning, and Redistribution within Plant Strata in Soybean

Introduction

Studying dry matter accumulation and nitrogen uptake, partitioning, and redistribution in soybean is crucial due to its direct impact on seed yield and overall plant productivity. Gaspar et al. (2017) highlighted the significance of understanding these processes, especially with the advancements in modern agricultural practices that have led to increased soybean yields. Moreover, the redistribution of nitrogen within soybean plants plays a compensatory role during seed filling when nitrogen uptake from soil and assimilation from the atmosphere may be limited (Sinclair & Wit, 1976; Egli et al., 1983; Miceli et al., 2000; Lumactud et al., 2022). Fundamental studies reported in the 1960's used old soybean lines with an average yield of around 1670 kg ha^{-1}. However, with the introduction of modern soybean lines, average yield has increased to 4421 kg ha^{-1} (Gaspar et al., 2017). Their results highlight greater remobilization efficiencies and late-season N uptake in conjunction with a greater nitrogen harvest index (NHI) to support higher yields per unit N uptake in current production systems. However, variation in dry matter accumulation and nitrogen uptake, partitioning, and redistribution by depth within the canopy has not been reported.

Dry matter accumulation and nitrogen uptake, partitioning, and removal are crucial factors determining soybean yield and quality. Bender et al. (2015) emphasized the significance of understanding these dynamics in current agricultural contexts, particularly considering modern production practices and genetics. They highlighted that the improved potential for dry matter production in modern soybean varieties has increased nutrient accumulation, underscoring the importance of studying nutrient uptake and partitioning. Moreover, Gaspar et al. (2017) highlighted the influence of modern production practices and genetics on soybean dry matter accumulation and nitrogen uptake, partitioning, and removal patterns and rates, emphasizing the need to understand these dynamics. Furthermore, Neto et al. (2021) found that high-yield soybean varieties exhibited differences in nutrient removal, indicating the need to comprehend the nutrient dynamics for optimizing yield.

Our study addresses a crucial gap in the current understanding of soybean dry matter accumulation and nitrogen uptake, partitioning, and removal with depth in the canopy. Previous studies, such as those by Hanway and Weber (1971) and Bender et al. (2015), have reported results based on whole plant yield at maturity. However, they have not comprehensively reported these dynamics through soybean growth and development and with depth in the canopy. Our study aims to fill this gap and provide valuable insights into these dynamics.

Materials and Methods

Field experiment

Field trials were conducted at the University of Nebraska-Lincoln, Campus Research Station in 2020 and 2021. The soil type at the farm is a Kennebec silt loam (fine-silty, mixed, super active, mesic Cumulic Hapludoll). The two varieties selected were based on commercial varieties in Nebraska that are known to differ in protein concentration (Carciochi et al., 2023). Pioneer P27A17X (MG 2.7) was selected for its lower protein concentration (33.5%), and Hoegemeyer LL2841 (MG 2.8) was selected for its greater protein concentration (35.7%). Three planting dates were used to establish crop development environments. The three planting dates were selected to be approximately 200 growing degree days (GDD) apart based on 20 years of weather data in Nebraska. Sowing dates occurred on April 27, May 18, June 8, 2020, April 27, May 18, and June 3, 2021, respectively, for early, mid, and late planting dates.

Soybeans were planted using a six-row planter with a 0.76-m row spacing. The target seeding rate was 432,250 seeds per hectare to minimize soybean branching. Weeds were manually controlled during the growing season until canopy closure. No insect or pathogen management was implemented in this study. Each experimental unit consisted of 20 rows of soybeans, which spanned 15.2 meters by 12.8 meters in length.

Plant biomass sampling, processing, and analysis

Above-ground biomass sampling was performed weekly from the vegetative stage until soybean maturity (R8). Growth stage was determined weekly by following Fehr and Caviness (1977). A 1-m row biomass subsample was harvested weekly. Subsample areas were at least 2 meters from the plot edge and 1 m between sampling areas to avoid edge effects. Each harvested row was 1 meter at the same row and 2 rows apart to avoid border effects. Whole plants were harvested from each subplot by clipping plants at the cotyledon node and bundling them for processing in the lab. A one-meter-long plastic net was placed in between rows within the center four rows of each experimental unit beginning at R1 to collect fallen leaves and petioles weekly to determine abscised leaf and petiole mass and N content. The two middle rows of each experimental unit were combine harvested to determine whole plot final yield at R8.

After subplot harvest, number of plants per subsample was counted and ten plants randomly selected to assess growth stage. All plants within subsample were then divided into five canopy layers, herein referred to as strata, based on the number of nodes. Strata one included nodes 1 – 5, two nodes 6 – 9, three nodes 10 – 13, four nodes 14 – 17, and five nodes 18+. Within each strata, plants were partitioned into stems, green leaves (lamina), petioles, pods, and yellow leaves, bagged separately and dried at 60 C to constant weight. From seed fill (R5) to maturity (R8), reproductive organs were separated into seed and pod walls and processed similarly. Final seed yield values are reported at 130 g kg^{-1} moisture content, except when analyzed with DM accumulation, in which case they are reported on a DM basis.

All dried plant organ samples were ground to pass through a 1-mm mesh screen using a Wiley Mill (Thomas Wiley Mills model 4). Seeds were ground using a coffee grinder to avoid contamination and oil loss. Nitrogen concentration was determined by combustion using Leco FP-528. Seed protein content was estimated as percent N concentration x 6.25 x seed mass on a dry matter basis and then converted to 13% moisture content (FAO, 2002). Oil concentration was determined using the accelerated solvent extraction (ASE) method (Matthaus & Bruhl, 2001) with modifications to the hexane evaporation process using the Genevac Rocket Synergy Evaporation System.

Seed oil content was calculated as percent oil concentration x seed mass on a dry matter basis and then converted to 13% moisture content.

Nitrogen redistribution from vegetative organs to the seed was calculated from R5 until maturity (R8) in the whole canopy and within strata using:

$$\text{Total N remobilization} = \text{N}_{\text{vegetative organs}} \text{ at R5} - \text{N}_{\text{vegetative organs}} \text{ at R8}$$

$$\text{Total N uptake} = \text{Total N}_{\text{seed}} \text{ at R8} - \text{Total N remobilization}$$

Where N in vegetative organs at R5 is the total nitrogen mass from leaves, petioles, stems, and pod walls at R5, N in vegetative organs at R8 is the total nitrogen mass from fallen leaves, leaves, petioles, stems, and pod walls at R8, and Total N in seed at R8 is the final nitrogen mass in the seed.

Experimental design and statistical analysis

The experiment was designed using a completely randomized design and a split-plot factorial with four replications for 2020 and 2021, respectively. The main plot consisted of planting dates, while soybean variety was used as subplots. In the first year, all replications were randomized within planting date with the assumption that the confounder effect was not present, and in the second year, all treatments were randomized in replicate blocks.

Statistical analysis was performed using PROC MIXED in SAS (SAS Institute, Cary, NC). The effect of planting date, variety, and their interactions on DM accumulation, DM harvest index (HI), N uptake, seed N concentration, and NHI at R8 was examined. Least squares means were compared using the Tukey grouping test at the

0.05 probability level. The statistical model included variety and planting date as fixed effects in 2020 and 2021 and planting date by replication as random effects in 2021.

Dry matter accumulation and N uptake, partitioning, and remobilization between various plant organs were modeled across the growing season using days after emergence (DAE). The figures were generated for DM and N to assess changes between different planting dates and varieties in 2020 and 2021 on plant strata and whole canopy profile. The figures were built in GraphPad Prism version 10.2.3 for Windows (GraphPad Software, Boston, Massachusetts, USA) using multiple spline curve options with smoothed data points, where all units are expressed on a dry-weight basis. A three-parameter logistic growth model described by Gaspar et al. (2017) was used to fit DM accumulation and N uptake on DAE for the whole plant and within each strata for each treatment. The derivative of this function was then used to plot biomass or N accumulation rate for the whole plant and each strata.

Results

Final dry matter accumulation was affected by the interaction of planting date and variety in 2021 but not in 2020, in which only planting date affected final above-ground dry matter (Table 2.1). Conversely, the interaction of planting date and variety significantly affected the yield, dry matter harvest index, total N uptake, N harvest index, seed N concentration, and seed N removal in 2020 and 2021. Late-planted Pioneer P27A17X showed greater DM harvest index, total N uptake, N harvest index, seed N concentration, and seed N removal in 2020 but not total N uptake (Tables 2.2 & 2.3). In 2021, early planted Hoegemeyer LL2841 had the greatest DM accumulation, total N uptake, seed N concentration, and seed N removal, while early planted Pioneer P27A17X had the greatest DM and N harvest index. Early and late-planting date treatments had greater dry matter accumulation compared to mid-planting dates in 2020.

Table 2.1 Analysis of covariance for yield, total dry matter (DM) accumulation, DM harvest index, total N uptake, N harvest index, seed N concentration, and seed N removal at growth stage R8.

Source	df	Yield	DM Accumulation	DM Harvest Index	Total N Uptake	N Harvest Index	Seed N Concentration	Seed N Removal
		-- P > F ---						
Year 2020								
PD	2	<.0001	0.0004	0.0003	<.0001	<.0001	<.0001	<.0001
Variety	1	0.0003	0.7083	0.1288	0.0005	0.5486	<.0001	<.0001
PD*Variety	2	<.0001	0.1520	0.0099	<.0001	<.0001	<.0001	<.0001
Residual	18							
Year 2021								
PD	2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Variety	1	0.9538	0.0239	0.0019	0.0291	<.0001	<.0001	0.0013
PD*Variety	2	<.0001	<.0001	0.0041	<.0001	<.0001	0.0157	<.0001
PD*Rep	9	0.1137	0.1947	0.2640	0.1147	0.2606	0.5718	0.0817
Residual	9							

Table 2.2 The least squares means for the interaction of planting date and variety on yield, total dry matter (DM) accumulation, and harvest index at growth stage R8.

PD	Variety	Yield (g m^{-2})*	DM accumulation (g m^{-2})*	DM Harvest Index*
Year 2020				
Early	Hoegemeyer LL2841	425.7 b	1091.0 a	38.9 b
Early	Pioneer P27A17X	412.2 b	1091.0 a	37.9 b
Mid	Hoegemeyer LL2841	317.5 d	889.3 b	34.6 b
Mid	Pioneer P27A17X	285.1 e	889.3 b	33.1 b
Late	Hoegemeyer LL2841	362.1 c	1000.0 a	38.2 b
Late	Pioneer P27A17X	485.1 a	1000.0 a	47.2 a
Year 2021				
Early	Hoegemeyer LL2841	451.2 a	1091.9 a	41.3 b
Early	Pioneer P27A17X	417.5 b	961.1 b	43.5 a
Mid	Hoegemeyer LL2841	328.3 d	846.8 c	38.8 c
Mid	Pioneer P27A17X	295.8 e	765.5 d	38.6 c
Late	Hoegemeyer LL2841	346.8 c	859.7 c	40.3 b
Late	Pioneer P27A17X	413.4 b	1009.0 b	41.0 b

* Tukey grouping for LS-means (Alpha=0.05). LS-means indicated with the same letter in the same column in the same year are not significantly different.

Table 2.3 The least squares means for the interaction of planting date and variety on total N uptake, N harvest index, seed N concentration, and seed N removal at growth stage R8.

PD	Variety	Total N Uptake (g m^{-2})*	N Harvest Index*	Seed N Concentration (%)*	Seed N removal (g m^{-2})*
Year 2020					
Early	Hoegemeyer LL2841	36.3 a	71.5 c	6.12 d	26.0 b
Early	Pioneer P27A17X	36.5 a	69.9 c	6.19 bc	25.5 b
Mid	Hoegemeyer LL2841	28.7 b	68.5 c	6.20 b	19.7 d
Mid	Pioneer P27A17X	27.3 b	64.5 d	6.17 bcd	17.6 e
Late	Hoegemeyer LL2841	29.3 b	75.7 b	6.13 cd	22.2 c
Late	Pioneer P27A17X	37.4 a	82.5 a	6.35 a	30.8 a
Year 2021					
Early	Hoegemeyer LL2841	36.7 a	81.4 b	6.62 a	29.9 a
Early	Pioneer P27A17X	31.6 b	85.2 a	6.45 c	26.9 b
Mid	Hoegemeyer LL2841	26.3 d	81.5 b	6.54 b	21.5 d
Mid	Pioneer P27A17X	25.7 d	73.3 c	6.36 d	18.8 e
Late	Hoegemeyer LL2841	25.8 d	84.3 a	6.28 e	21.8 d
Late	Pioneer P27A17X	30.1 c	84.7 a	6.16 f	25.4 c

* Tukey grouping for LS-means (Alpha=0.05). LS-means indicated with the same letter in the same column in the same year is not significantly different.

Dry matter accumulation

The R1 growth stage occurred approximately one-third of the way through the growing season for most planting dates in the whole canopy profile (Figure 2.1 – 2.6). However, the total dry matter accumulation at this point only accounted for 6 – 8 (57 – 90 g m^{-2}), 9 – 18 (77 – 166 g m^{-2}), and 11 – 21% (104 – 190 g m^{-2}) of total dry matter accumulation at maturity for early, mid, and late planting dates, respectively in both years. This accumulation was distributed only in strata 1 and 2. Furthermore, 50% dry matter accumulation of the whole plant was attained at the R3 growth stage and ranged from 488 – 547, 386 – 461, and 435 – 527 g m^{-2} for early, mid, and late planting dates, respectively. By the time of 50% whole plant dry matter accumulation, most plants contained four strata, which is ~80% of the total node number in the whole canopy.

Earlier planting dates tend to lead to longer periods of plant growth and a slower rate of DM accumulation in the whole canopy profile (Figure 2.7 & 2.8). Until the R1 growth stage, the early planting date displayed a whole plant DM accumulation rate < 10 g m^{-2} day^{-1}, while mid and late planting dates displayed around 15 g m^{-2} day^{-1} in 2020, and a slightly lower trend for early and mid-planting date in 2021. Since the earliest planting date had a slow DM accumulation at the beginning of the growing season, the maximum whole plant DM accumulation rate was attained around the R3 growth stage, ranging from 19.5 – 22.4 g m^{-2} day^{-1}. Meanwhile, mid and late-planting dates attained maximum DM accumulation rate before the R3 growth stage, ranging from 20.2 – 26.2 and 24.3 – 30.7 g m^{-2} day^{-1}, respectively.

The dry matter (DM) accumulation within plant strata varies with planting date and growth stage (Figure 2.1 – 2.6). At the R1 growth stage, DM accumulation occurred in strata 1 and 2. Following this, DM accumulation in strata 3 occurred from the R2 growth stage for all planting dates. Additionally, DM accumulation in strata 4 occurred from the R3 growth stage for early and mid-planting dates, and at the R4 stage for late planting dates. Finally, DM accumulation in strata 5 occurred between the R4 and R5 growth stages for all planting dates in both years.

The peak DM accumulation rate within each strata was attained at different growth stages. The peak DM accumulation rate in strata 1 was reached after the R1 growth stage for the early planting date and at the vegetative stage for the mid and late-planting dates, ranging from 2.8 – 8.4 g m^{-2} day^{-1}. The peak DM accumulation rate in strata 2 was attained right before or after the R2 growth stage for all planting dates, ranging from 6.9 – 17.3 g m^{-2} day^{-1}. The peak DM accumulation rate in strata 3 was attained around the R4 growth stage except for mid-planting date in 2020, where it was attained before the R3 growth stage, ranging from 9.2 – 16.0 g m^{-2} day^{-1}. The peak DM accumulation rate in strata 4 was reached after the R5 growth stage except for the mid-planting date in 2020, where it was reached before the R5 growth stage (4.5 – 8.3 g m^{-2} day^{-1}). The peak DM accumulation rate in strata 5 was reached after R5 or during R6 (0.4 – 6.9 g m^{-2} day^{-1}). Strata 2 and 3 contributed to the top two greatest DM accumulation rates in all different planting dates and varieties in 2020 and 2021.

Dry matter partitioning

In the vegetative stage, leaves dominated organ growth, followed by stems and petioles. Right before the beginning of flowering (R1), 54, 51, and 48% of the DM accumulation was partitioned to leaves, 27, 27, and 30% to stems, and 19, 23, and 22% to petioles in the early, mid, and late planting dates, respectively in 2020. Even though the early and late-planting dates had higher DM accumulation compared to the mid-planting date in 2020, biomass partitioning varied among planting dates. This trend also was seen in 2021, where DM accumulation partitioned to the leaves ranged from 46 – 55%, to stems from 27 – 35%, and to petioles from 14 – 22% for all combinations of planting dates and varieties in 2021 (Figure 2.1 – 2.6, Table 2.3).

The growth of vegetative organs reached a maximum and ceased at different growth stages for all treatments in the whole canopy profile (Figure 2.1 – 2.6) before remobilizing to pods and seeds. While there were no differences among varieties for DM accumulation in 2020, each variety showed a different pattern for the maximum accumulation of vegetative organs at different planting dates (Figure 2.1 – 2.3). For instance, Hoegemeyer LL2841 reached maximum DM for leaves and petioles at the R3, R4, and R6 stages for early, mid, and late-planting dates, respectively. Pioneer P27A17X attained maximum DM for leaves and petioles at the R5, R4, and R5 stages for early, mid, and late-planting dates, respectively. Maximum DM for stems was reached at the R6 stage for all treatments except Pioneer P27A17X at the late planting date, which was attained at the R5 stage.

A planting date by variety interaction for DM accumulation in 2021 resulted in different patterns in when the maximum DM accumulation occurred. Hoegemeyer LL2841 attained maximum DM for leaves and petioles at the R4, R3, and R6 stages for early, mid, and late-planting dates, respectively, except maximum DM for petioles at mid-planting dates was reached at the R5 stage. On the other hand, Pioneer P27A17X reached maximum DM for leaves and petioles at the R5 stage at all planting dates. The maximum stem DM accumulation in 2021 was similar to that in 2020 for all treatments.

Strata 1. Within strata 1, Hoegemeyer LL2841 and Pioneer P27A17X reached maximum leaf and petiole DM at the R3 stage for the early planting date in 2020. On the other hand, both varieties reached maximum leaf DM at the R1 stage, and for petioles at the R2 stage for mid and late-planting dates, except that Hoegemeyer LL2841 reached maximum leaf and petiole DM at the R2 stage in the late-planting date. The maximum DM of stems showed the same pattern as the whole plant profile, which was attained at the R6 stage for all planting dates and varieties. A different pattern was shown for the 2021 growing season. The leaf DM reached a maximum at the R2 stage, and petiole DM reached a maximum at the R3 stage in the early planting date for both varieties. Leaf and petiole DM reached a maximum at the same R2 stage in the mid-planting date for both varieties. In contrast, at late-planting, Hoegemeyer LL2841 reached the maximum DM for leaves and petioles at the R2 stage, while Pioneer P27A17X reached the maximum DM for leaves at the R1 stage and for petioles at the R2 stage. The same pattern was shown for the stems as in the whole canopy profile, in which stem DM reached a maximum at the R6 stage for all treatments.

Strata 2. Variation in maximum DM of the various organ groups was shown in 2020. Hoegemeyer LL2841 reached a maximum leaf DM at the R3 stage for early and mid-planting dates and at the R2 stage for the late-planting date. Pioneer P27A17X reached a maximum leaf DM at R5, R2, and R4 for early, mid, and late-planting dates, respectively. Maximum petiole DM was attained at R4, R3, and R5 for Hoegemeyer LL2841 and at R5, R3, and R5 for Pioneer P27A17X at early, mid, and late-planting dates, respectively. Maximum stem DM was reached at the R6 stage for all treatments. However, results were slightly different in 2021. Most treatments reached a maximum leaf DM at the R3 stage, except for Hoegemeyer LL2841, which reached a maximum leaf DM at the R5 stage in the late-planting date. All varieties reached maximum petiole DM at the R3 stage in the early and mid-planting dates and at the R5 stage for the late-planting date. In all treatments, the same pattern was shown for the maximum DM of stems in both years, occurring at the R6 stage.

Strata 3. In the middle of the plant canopy, Hoegemeyer LL2841 reached maximum leaf and petiole DM at the R6 and R4 stage for early and mid-planting dates, but at R6 and R5, respectively, for the late planting date in 2020. However, for all planting dates, Pioneer P27A17X reached the maximum DM for leaves, petioles, and stems at the R6 stage. In the 2021 growing season, leaves and petioles reached maximum DM at the R5 stage for both varieties in early and mid-planting dates. Late-planted Hoegemeyer LL2841 attained maximum leaf and petiole DM at the R6 stage, and Pioneer P27A17X at the R5 and R6 for leaves and petioles, respectively. Maximum DM of stems was attained at the R6 stage for all treatments.

Strata 4 and 5. The top part of the soybean canopy, represented by strata 4 and strata 5, showed the same pattern for all vegetative organs for maximum DM except Pioneer P27A17X at the late-planting date in 2020 and Hoegemeyer LL2841 at the mid-planting date in 2021. The maximum stem DM was reached at the R7 stage for Pioneer P27A17X, and the maximum leaf DM was reached at the R5 stage for Hoegemeyer LL2841. However, for other combinations of planting date and variety, maximum leaf, petiole, and stem DM was reached by the R6 stage.

Harvest Index

Harvest index, reported on a percentage basis, was affected by the interaction of planting date and variety in both years (Table 2.3). In the 2020 growing season, late planted Pioneer P27A17X showed the highest harvest index (47%) compared to all other treatments, having harvest index between 34 – 39%. In the 2021 growing season, early planted Pioneer P27A17X showed the highest harvest index (44%), followed by both varieties in the late planting date and early planted Hoegemeyer LL2841 (40 - 41%).

Lowest harvest index occurred for both varieties in the mid-planting date treatment in 2021 (39%).

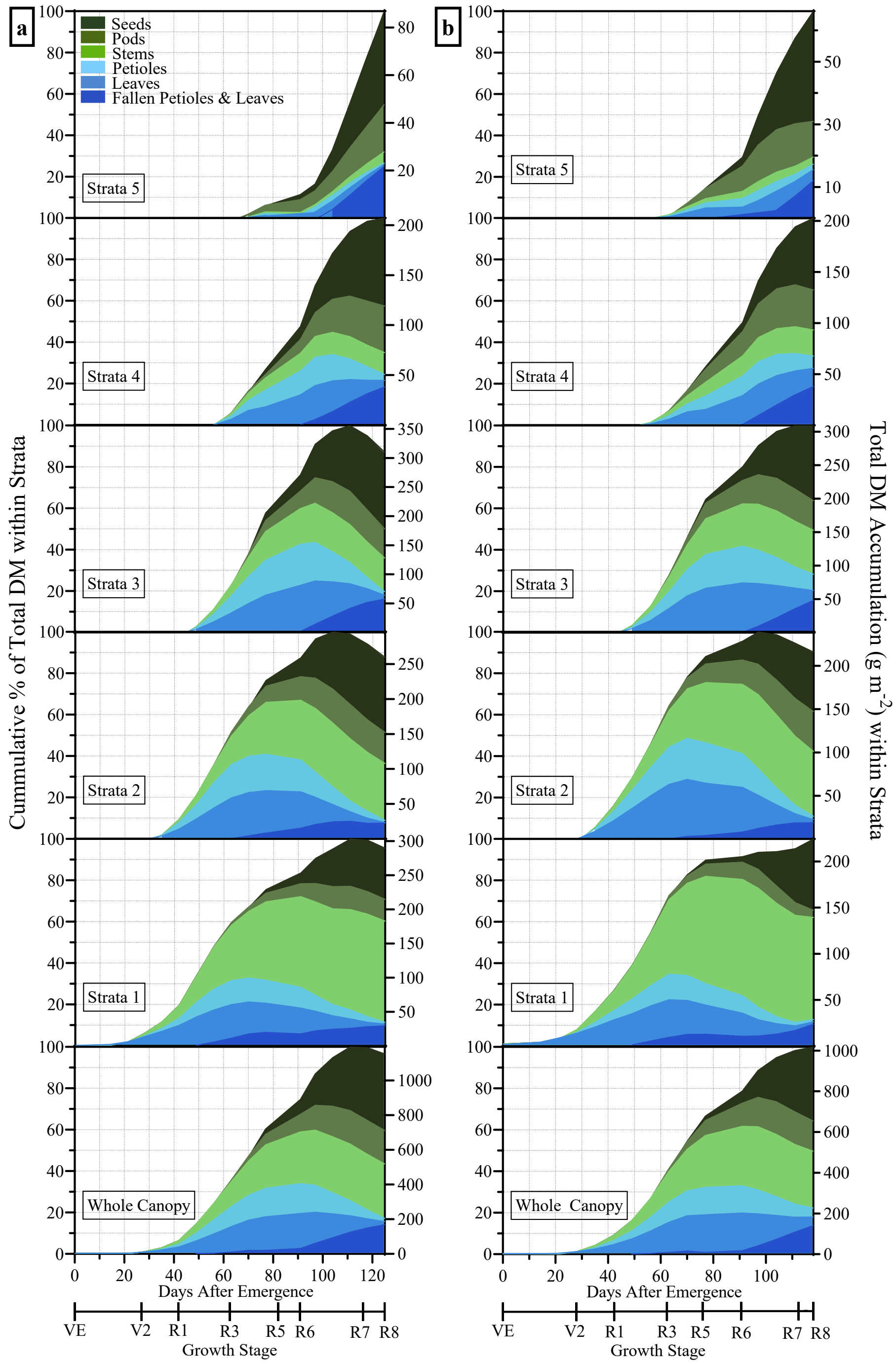

Figure 2.1 Dry matter (DM) accumulation and partitioning across the 2020 growing season for early planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+. (a) represents a high-protein-concentration cultivar (Hoegemeyer LL2841), and (b) represents a low-protein-concentration cultivar (Pioneer P27A17X).

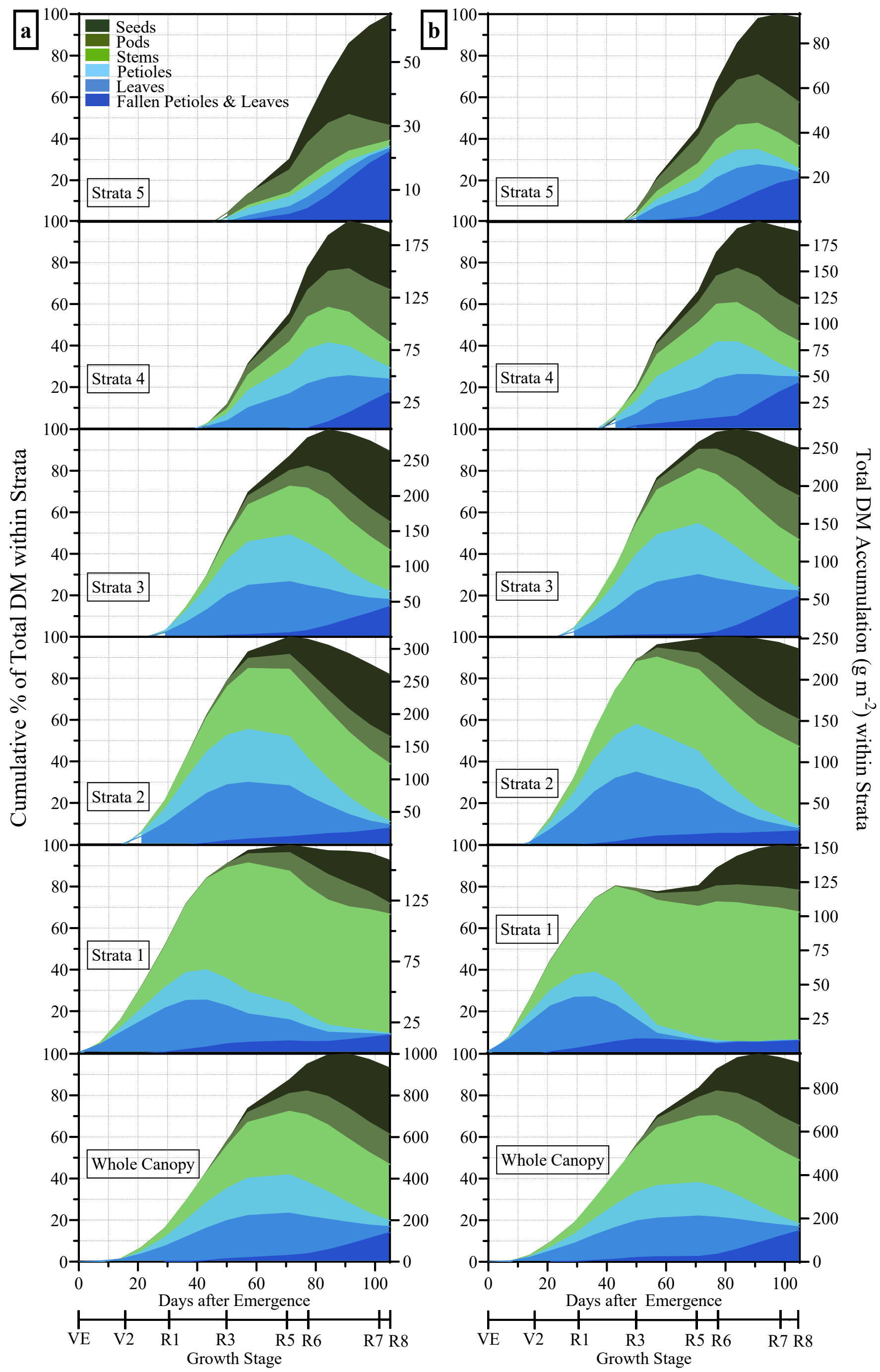

Figure 2.2 Dry matter (DM) accumulation and partitioning across the 2020 growing season for mid-planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+. (a) represents a high-protein-concentration cultivar (Hoegemeyer LL2841), and (b) represents a low-protein-concentration cultivar (Pioneer P27A17X).

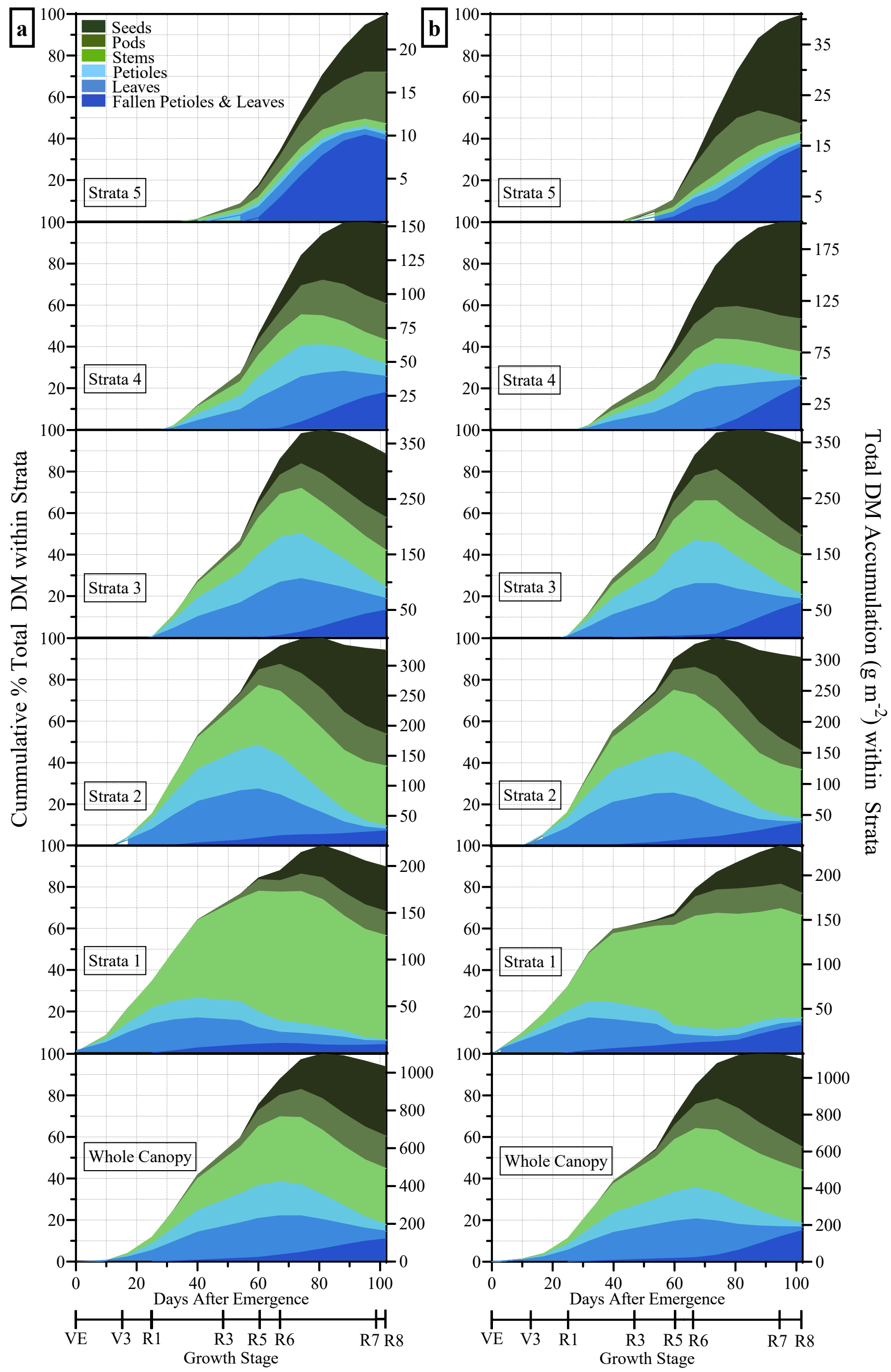

Figure 2.3 Dry matter (DM) accumulation and partitioning across the 2020 growing season for late-planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+. (a) represents a high-protein-concentration cultivar (Hoegemeyer LL2841), and (b) represents a low-protein-concentration cultivar (Pioneer P27A17X).

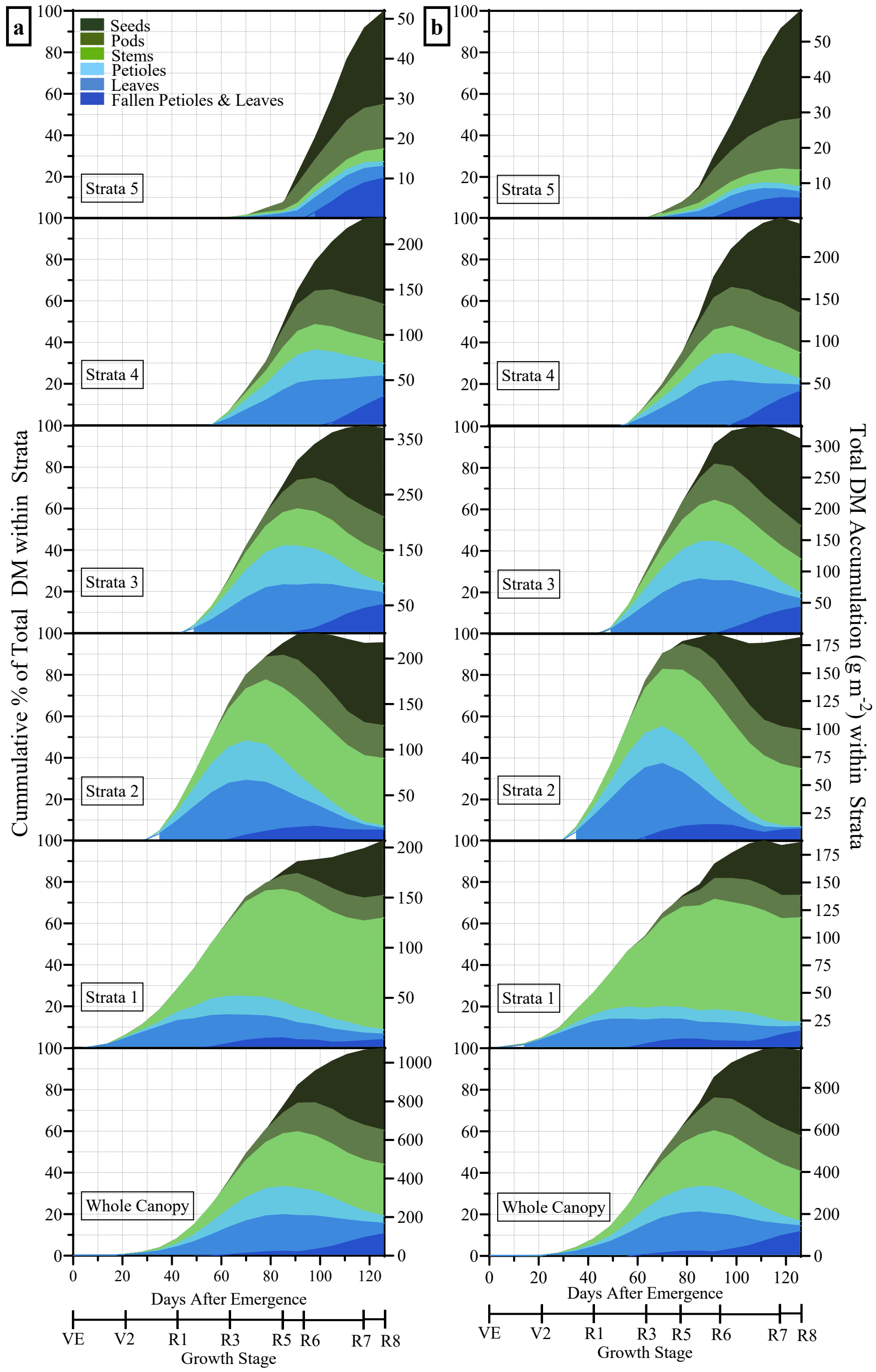

Figure 2.4 Dry matter (DM) accumulation and partitioning across the 2021 growing season for early planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+. (a) represents a high-protein-concentration cultivar (Hoegemeyer LL2841), and (b) represents a low-protein-concentration cultivar (Pioneer P27A17X).

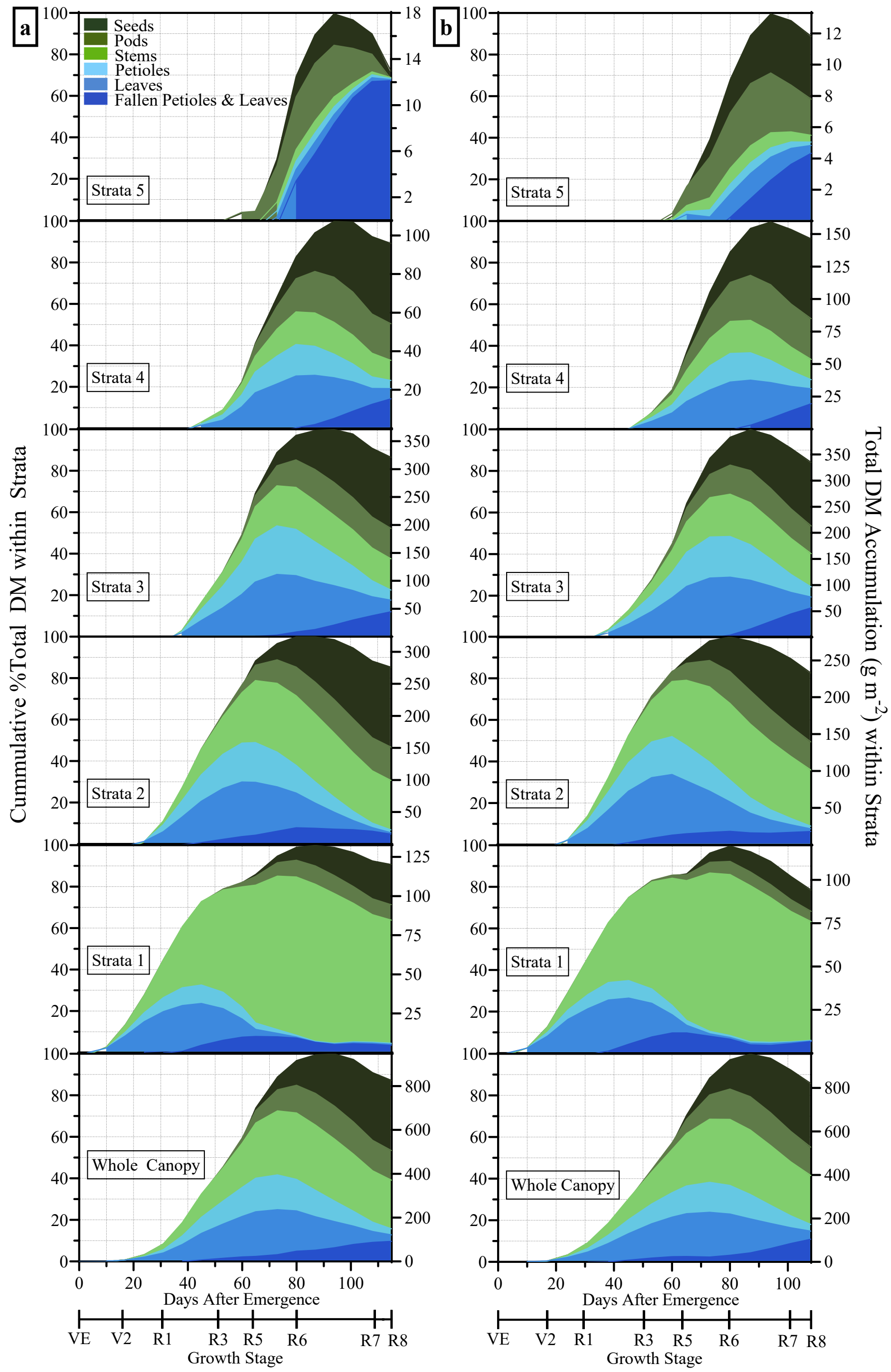

Figure 2.5 Dry matter (DM) accumulation and partitioning across the 2021 growing season for mid-planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+. (a) represents a high-protein-concentration cultivar (Hoegemeyer LL2841), and (b) represents a low-protein-concentration cultivar (Pioneer P27A17X).

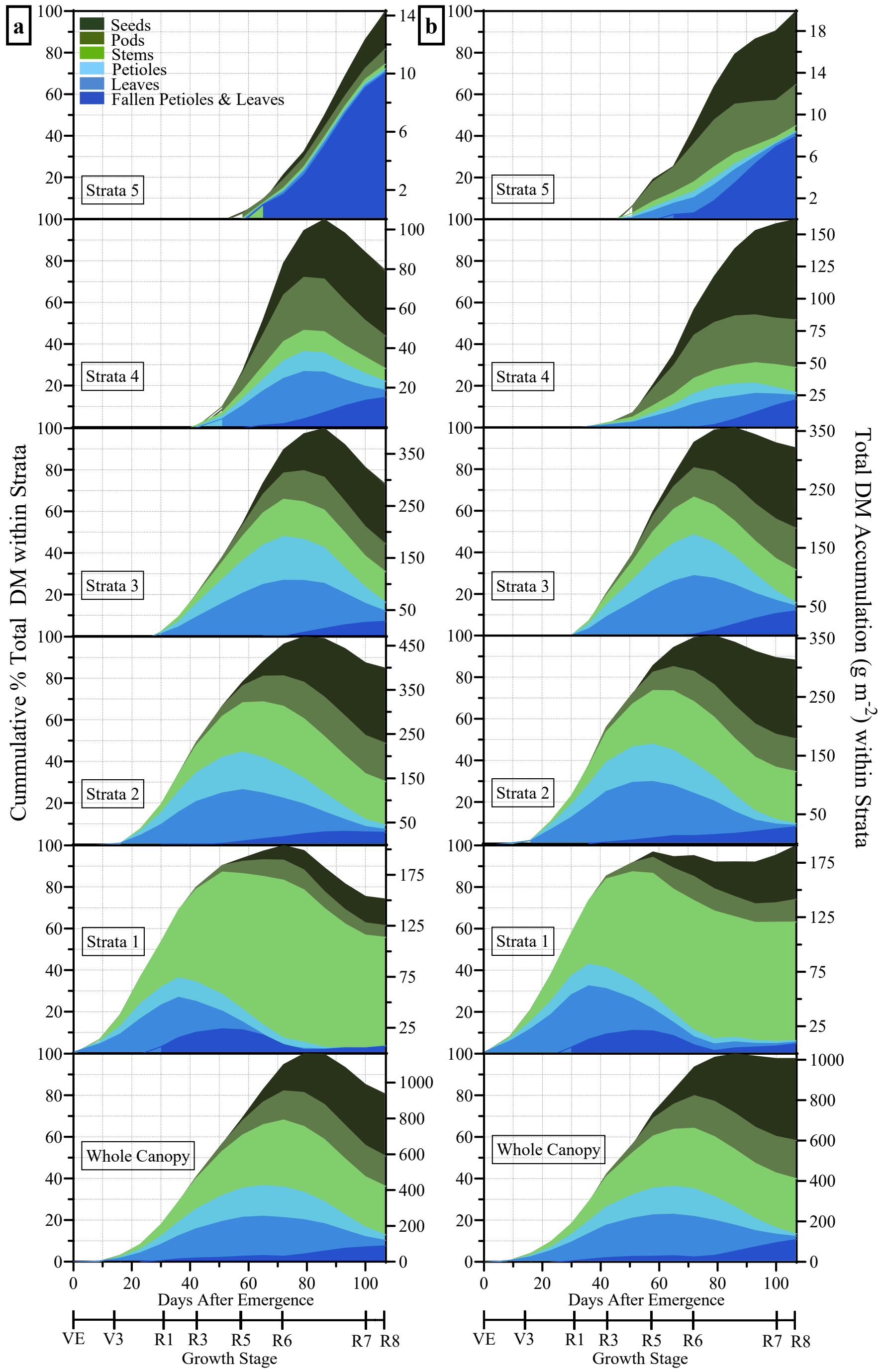

Figure 2.6 Dry matter (DM) accumulation and partitioning across the 2021 growing season for late-planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+. (a) represents a high-protein-concentration cultivar (Hoegemeyer LL2841), and (b) represents a low-protein-concentration cultivar (Pioneer P27A17X).

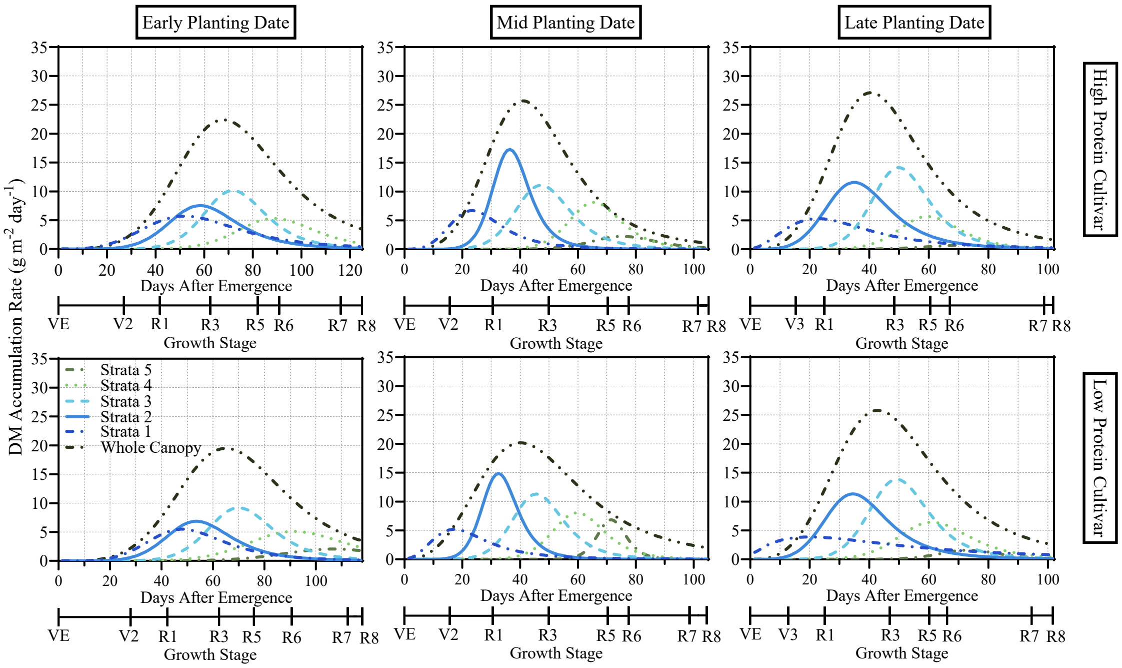

Figure 2.7 Dry matter (DM) accumulation rate across the 2020 growing season for early, mid, and late-planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+.

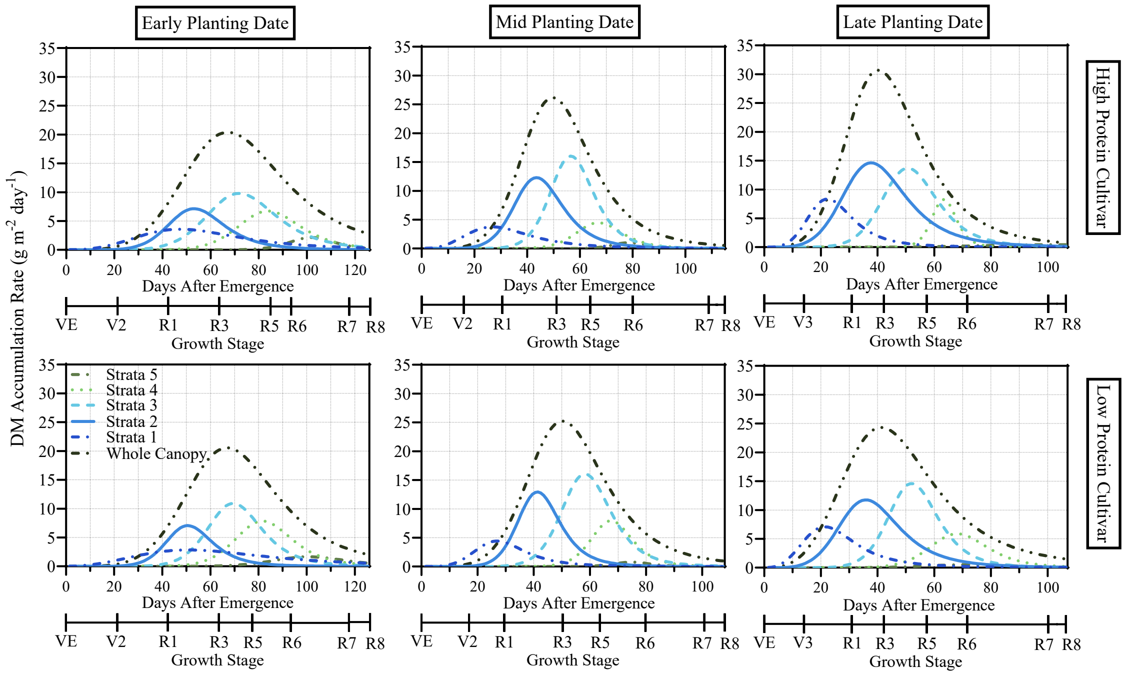

Figure 2.8 Dry matter (DM) accumulation rate across the 2021 growing season for early, mid, and late-planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+.

Nitrogen uptake

Planting date by variety interaction affected soybean nitrogen uptake in both years (Table 2.3). In the 2020 growing season, both varieties grown in the early planting date and Pioneer P27A17X grown in the late planting date resulted in the greatest whole plant nitrogen uptake, ranging from 36.3 – 37.4 g N m^{-2}. Other treatments resulted in nitrogen uptake between 27.3 – 29.3 g N m^{-2}. Early planted Hoegemeyer LL2841 resulted in the highest nitrogen uptake (36.7 g N m^{-2}) in 2021. Early and late planted Pioneer P27A17X had the next greatest nitrogen uptake of 31.6 and 30.1 g N m^{-2}, respectively. At the same time, the remaining treatments resulted in the lowest nitrogen uptake at the rate of 25.7 – 26.3 g N m^{-2}.

Whole plant nitrogen uptake at one-third of the way through the growing season differed slightly among planting dates in both years (Figure 2.9 – 2.14). The fraction of total N uptake at this stage was 7.8 – 8.2% (2.9 – 3.0 g N m^{-2}), 19.1 – 27.3% (5.5 – 7.5 g N m^{-2}), and 13.4 – 14.2% (4.0 – 5.3 g N m^{-2}) for early, mid, and late-planting dates, respectively in 2020. The fraction of total N uptake at R1 was 7.6 – 10.0% (2.4 – 3.7 g N m^{-2}), 11.5 – 12.7% (3.0 – 3.3 g N m^{-2}), and 26.2 – 26.8% (7.0 – 7.9 g N m^{-2}) for early, mid, and late-planting dates, respectively, in 2021. The time to 50% total N uptake differed from DM accumulation, attained between the R2 and R4 growth stages, depending on the planting date and variety.

At the beginning of the growing season, the earliest planting dates showed a longer lag phase where the N uptake rate was low in the whole canopy profile in 2020 (Figure 2.15). However, the mid and late-planting dates showed a shorter lag phase. For example, in the first 20 days after emergence, the early planting date had an N uptake rate of 0.032 and 0.042 g m^{-2} day^{-1} for Hoegemeyer LL2841 and Pioneer P27A17X, respectively. In contrast, mid and late-planting dates showed an N uptake rate range of 0.030 – 0.109 g m^{-2} day^{-1} in the ten days after emergence for both varieties. A different N uptake rate pattern was shown in 2021 (Figure 2.16). The longer lag phase was seen in the early and mid-planting date (0.021 – 0.065 g m^{-2} day^{-1}) for both varieties, while the late-planting date showed a shorter lag phase (0.038 – 0.078 g m^{-2} day^{-1}) for both varieties. Furthermore, the maximum N uptake rate was obtained at the R3 stage for the early planting date (0.550 – 0.642 g m^{-2} day^{-1}) in both years, whereas the mid and late-planting dates reached maximum N uptake rate at the R2 stage (0.578 – 0.771 and 0.675 – 0.822 g m^{-2} day^{-1}, respectively).

The peak of the total N uptake rate within the strata differed from that of the maximum DM accumulation rate (Figure 2.15 & 2.16). All treatments reached the maximum N uptake rate within strata one during late vegetative stages except for the early planting date in 2020, which peaked at R2 and R1. Strata 2 reached the maximum N uptake rate at the R1 and R2 stages for all treatments in both years. This was followed by strata 3, which reached maximum total N uptake at the R2 to R3 and R3 to R4 for 2020 and 2021, respectively. Strata 4 reached maximum N uptake at R4 to R5 and R5 for 2020 and 2021, respectively. Then, strata 5 reached the maximum total N uptake rate at R5 to R6 in all treatments.

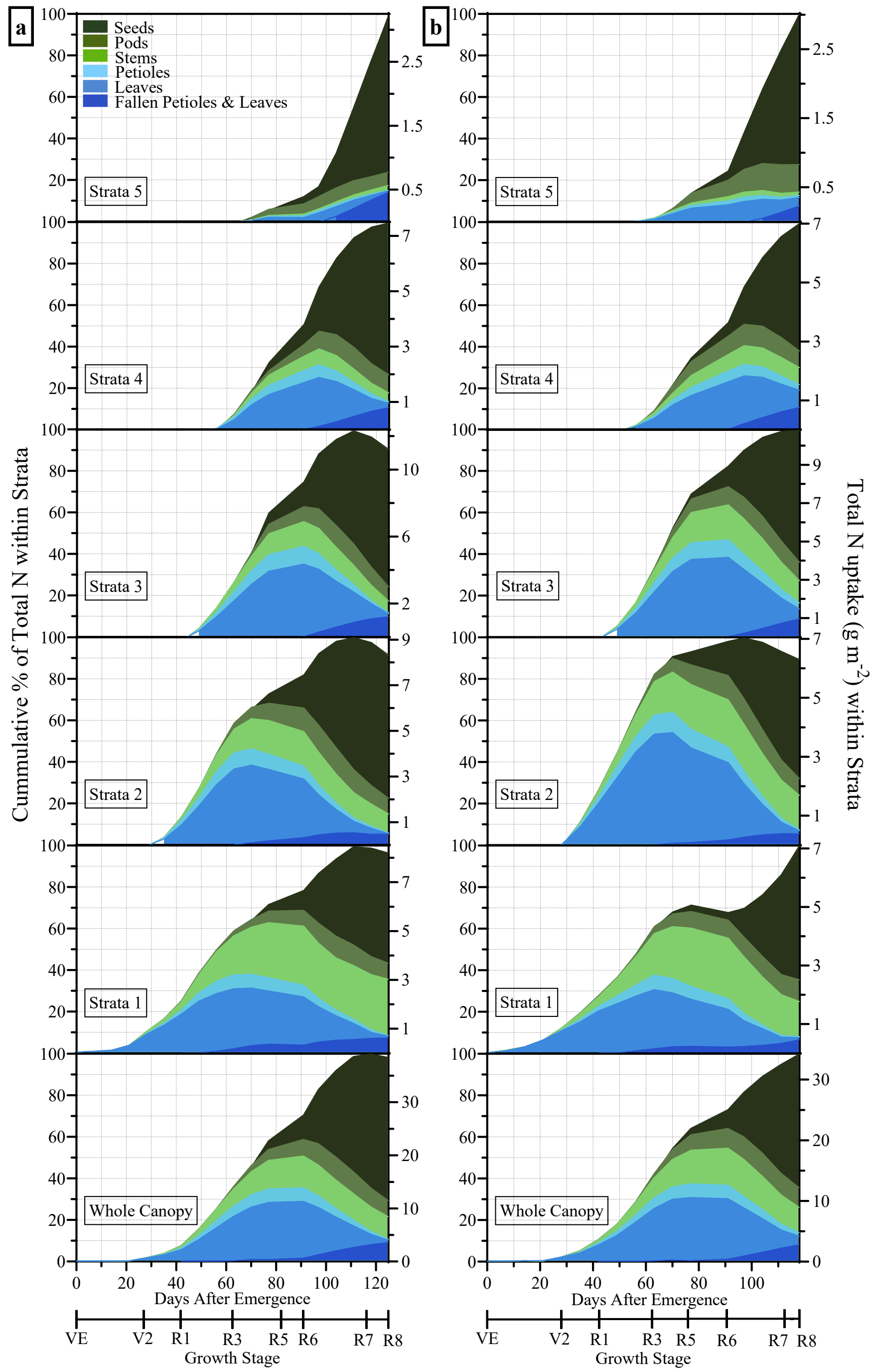

Figure 2.9 Total N uptake and partitioning across the 2020 growing season for early planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+. (a) represents a high-protein-concentration cultivar (Hoegemeyer LL2841), and (b) represents a low-protein-concentration cultivar (Pioneer P27A17X).

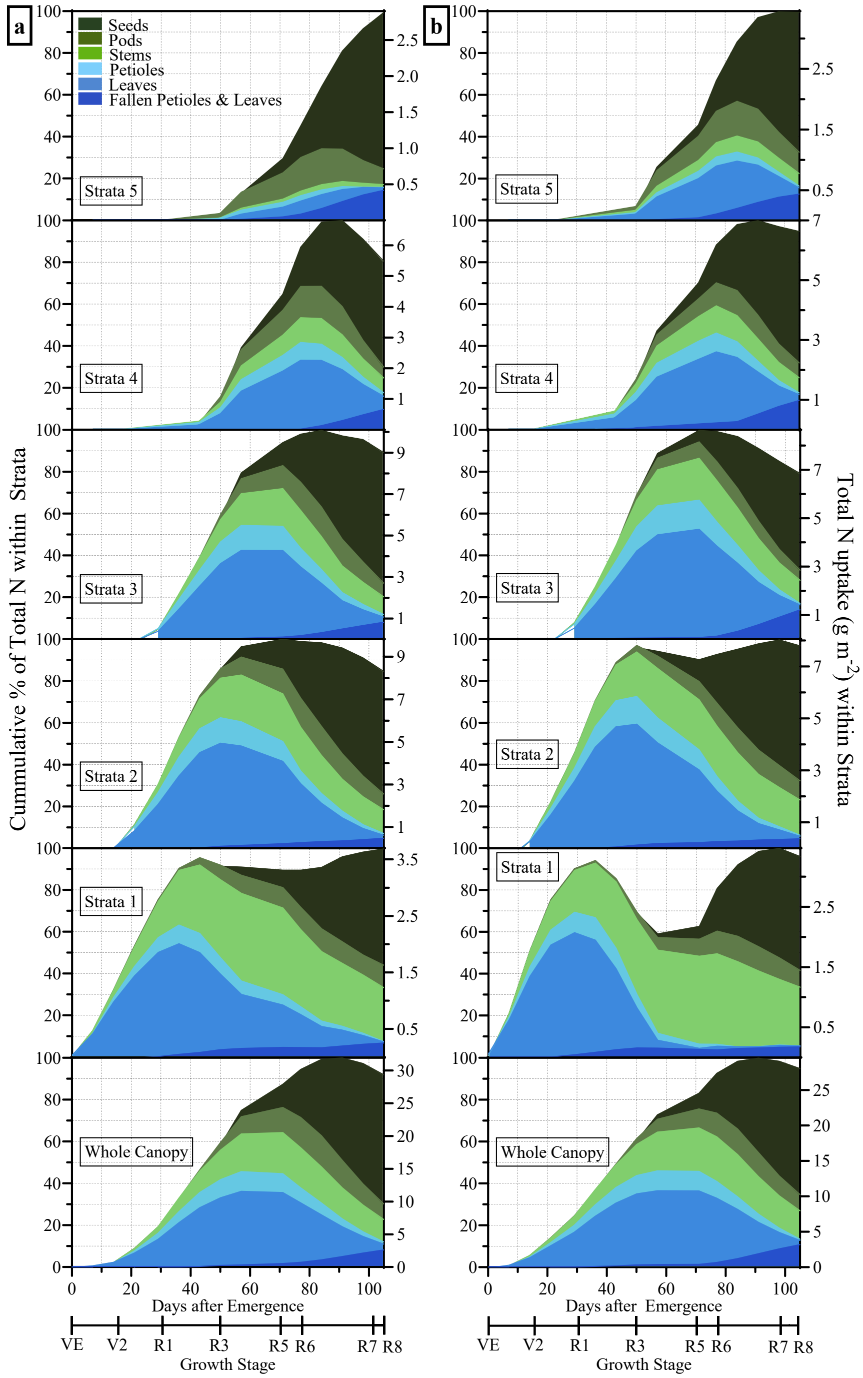

Figure 2.10 Total N uptake and partitioning across the 2020 growing season for mid planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+. (a) represents a high-protein-concentration cultivar (Hoegemeyer LL2841), and (b) represents a low-protein-concentration cultivar (Pioneer P27A17X).

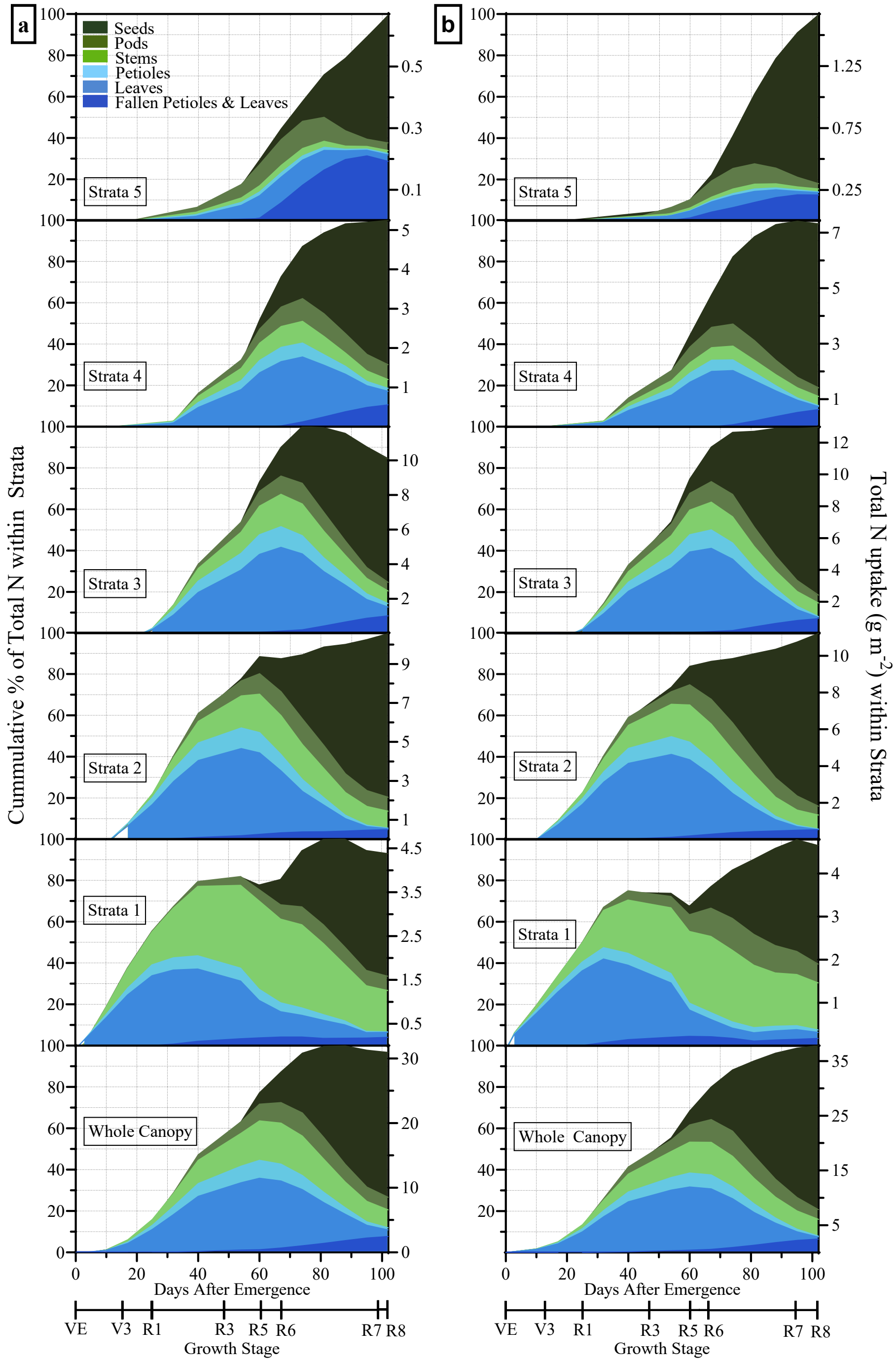

Figure 2.11 Total N uptake and partitioning across the 2020 growing season for late planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+. (a) represents a high-protein-concentration cultivar (Hoegemeyer LL2841), and (b) represents a low-protein-concentration cultivar (Pioneer P27A17X).

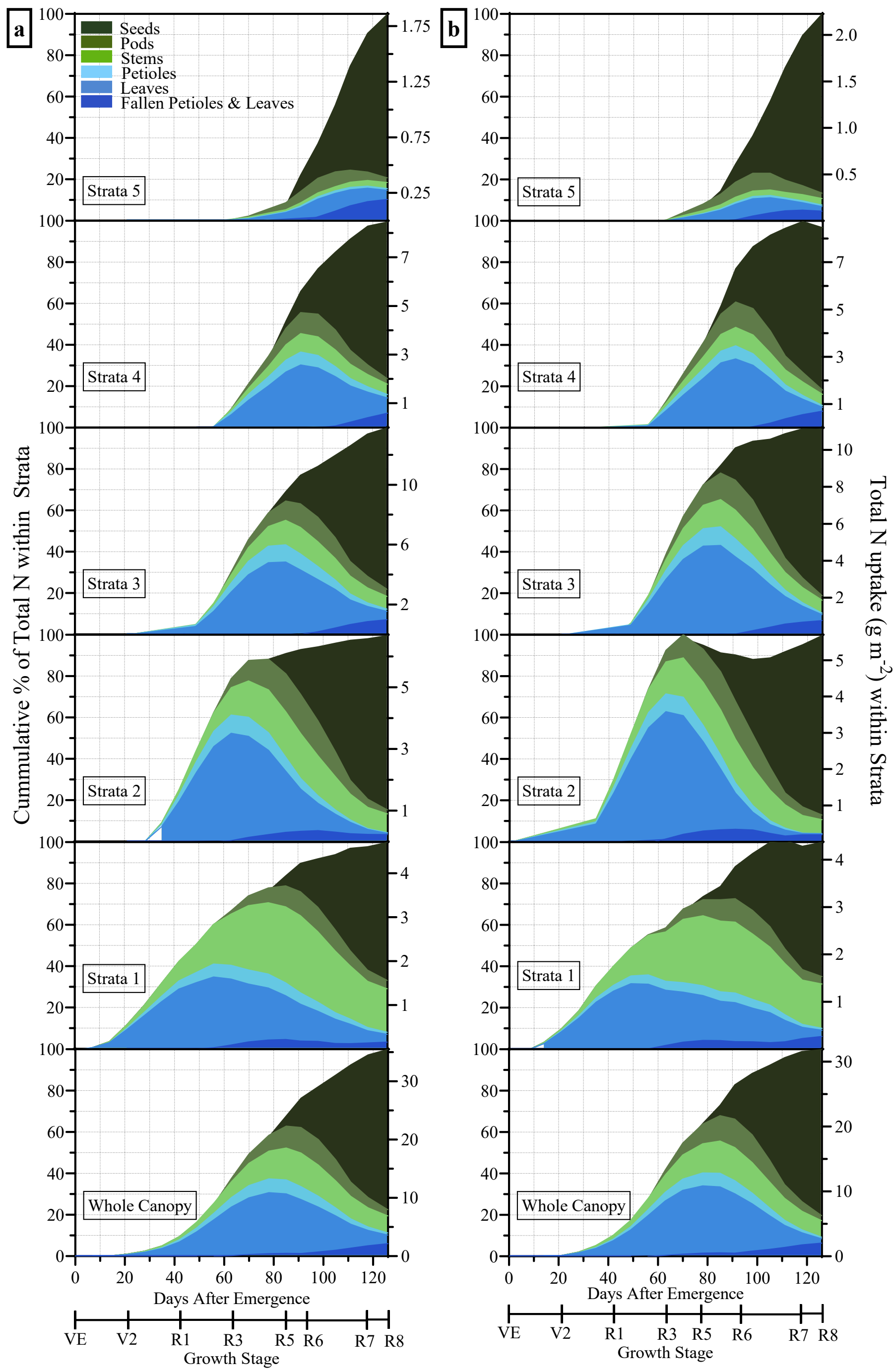

Figure 2.12 Total N uptake and partitioning across the 2021 growing season for early planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+. (a) represents a high-protein-concentration cultivar (Hoegemeyer LL2841), and (b) represents a low-protein-concentration cultivar (Pioneer P27A17X).

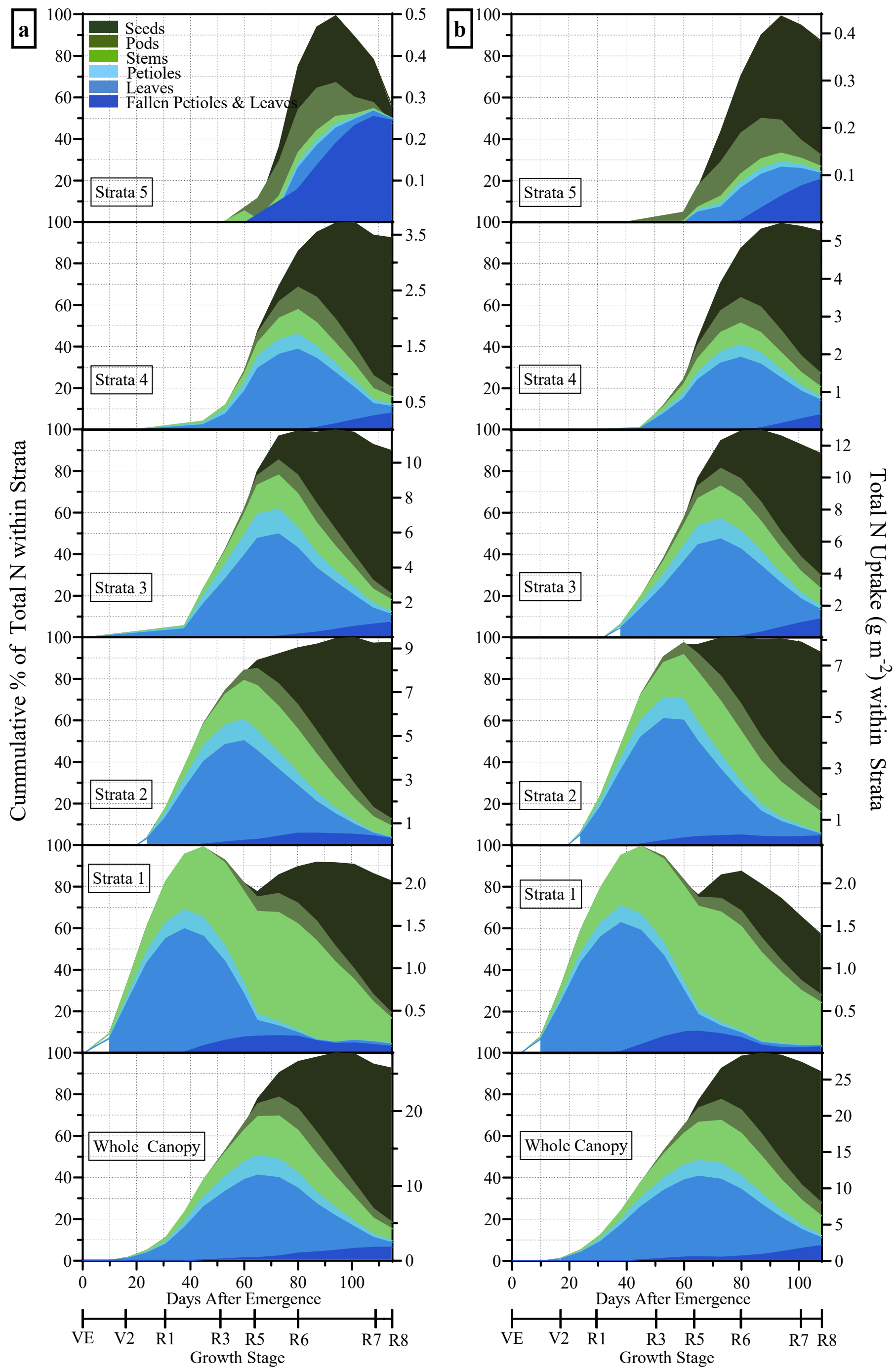

Figure 2.13 Total N uptake and partitioning across the 2021 growing season for mid-planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+. (a) represents a high-protein-concentration cultivar (Hoegemeyer LL2841), and (b) represents a low-protein-concentration cultivar (Pioneer P27A17X).

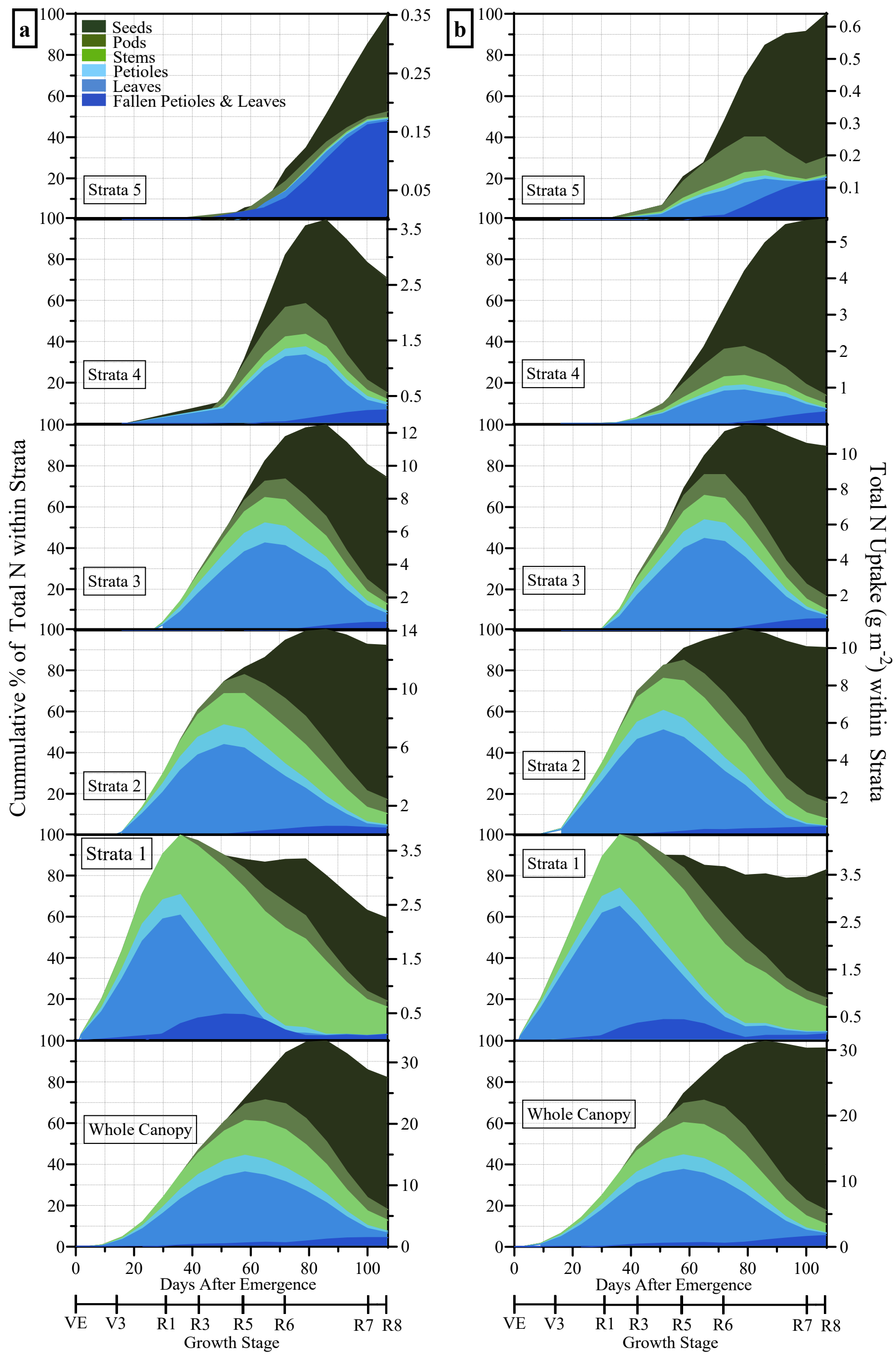

Figure 2.14 Total N uptake and partitioning across the 2021 growing season for late-planted soybeans separated by strata and for the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+. (a) represents a high-protein-concentration cultivar (Hoegemeyer LL2841), and (b) represents a low-protein-concentration cultivar (Pioneer P27A17X).

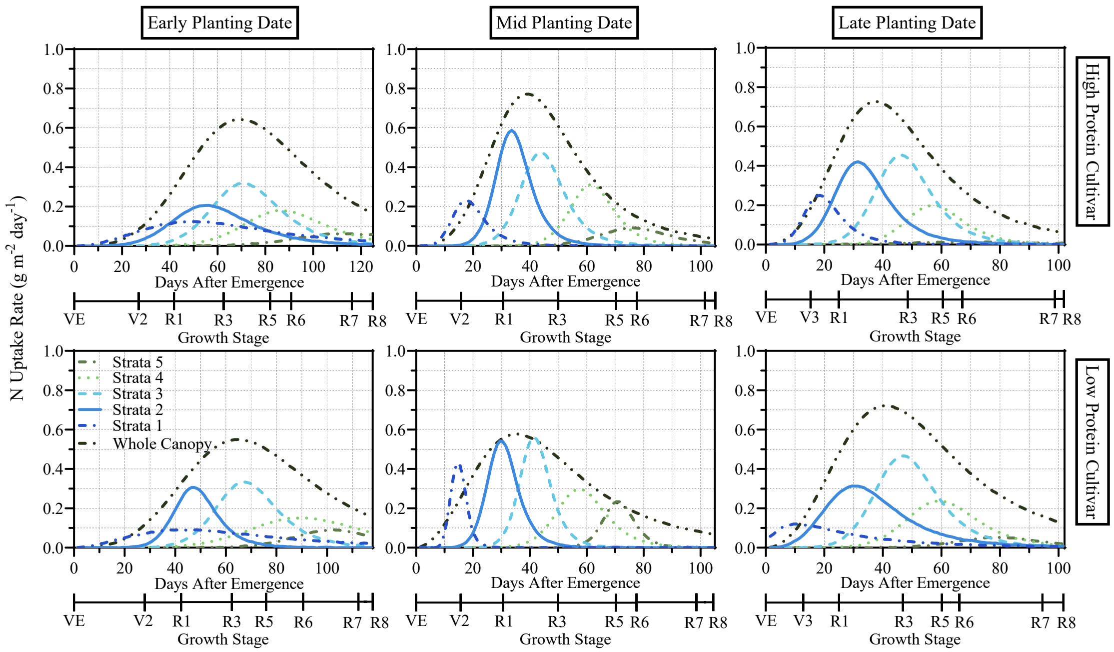

Figure 2.15 Nitrogen uptake rate across the 2020 growing season for early, mid, and late-planted soybeans separated by strata and the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+.

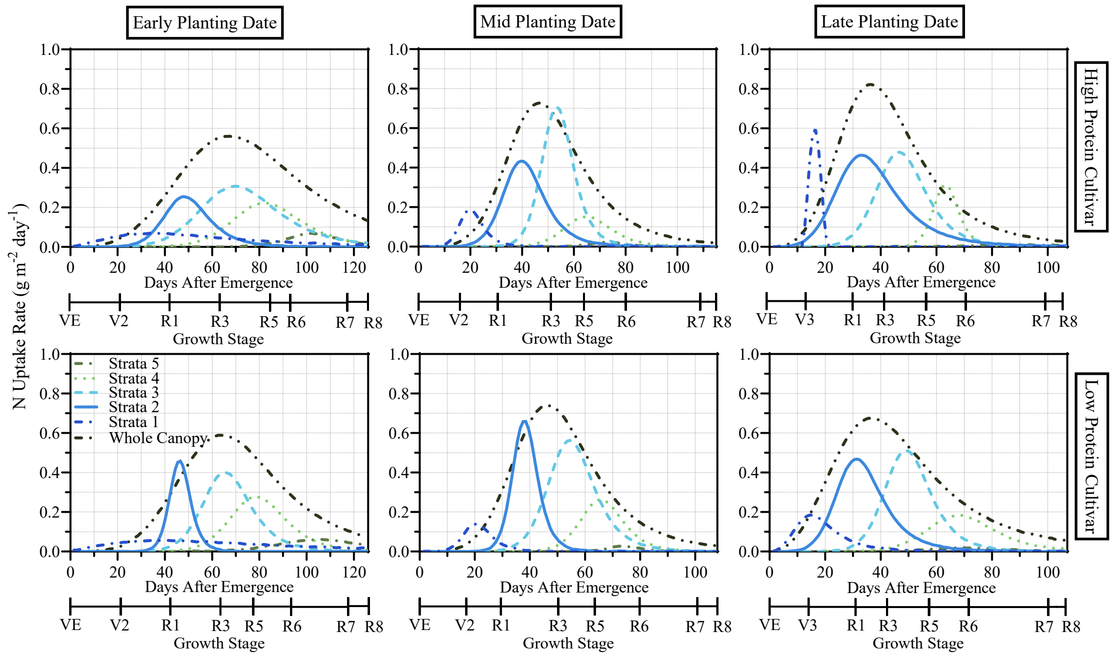

Figure 2.16 Nitrogen uptake rate across the 2021 growing season for early, mid, and late-planted soybeans separated by strata and the whole canopy. Strata 1 includes nodes 1-5, strata 2 nodes 6-9, strata 3 nodes 10-13, strata 4 nodes 14-17, and strata 5 nodes 18+.

Nitrogen partitioning

The remobilization of nitrogen content in plants shifts from one organ to another throughout the plant in the whole plant profile (Figure 2.9 – 2.14). After reaching the maximum N content in vegetative organs, the nitrogen was remobilized to the closest organ before it went to seed. For instance, leaves reach the maximum nitrogen content earlier, followed by petioles and stems. After stems reached maximum N content, the nitrogen content of the seed increased significantly. In the beginning of seed fill (R5), total N contained in the leaves, petioles, stems, and pods ranged from 39.4 – 44.2, 10.3 – 11.8, 22.7 – 27.9, and 10.7 – 19.2% in 2020, respectively, except for N in leaves of Pioneer P27A17X (51.8%). The N not redistributed was lost to fallen leaves and petioles, which ranged from 1.8 - 3.3% of the total N. A different trend was seen in the 2021 growing season, especially for N partitioned to the leaves. Nitrogen in leaves, petioles, stems, and pods ranged from 48.4 – 51.7, 9.9 – 13.5, 22.6 – 25.5, and 6.6 – 22.4%, respectively, except for the leaves of Hoegemeyer LL2841 (41.3%). The portion of N lost in fallen leaves and petioles ranged from 2.7 – 4.1% of total N taken up in 2021.

After R5, N uptake was partitioned mainly to the seed, and N stored in vegetative organs such as leaves, petioles, stems, and pod walls began rapidly remobilizing to the seed. From R5 to maturity in the whole plant, 10.6 – 11.4, 12.0 – 13.3, and 16.5 – 19.2 g N m^{-2} (50.6 - 50.7, 55.5 – 59.2, and 69.8 - 74.2% of total vegetative N) was remobilized to the seed for early, mid, and late-planting date in 2020, respectively. In 2021, the plants were more efficient in partitioning N to the seed, where 16.3 – 17.5, 14.4 – 15.8, and 16.0 – 16.1 g N m^{-2} (or 71.9 – 76.6, 67.5 – 76.5, and 77.6 – 79.4% of total vegetative N) was remobilized to the seed for the early, mid, and late-planting date, respectively.

Strata 1. General trends showed that most of the nitrogen reserves in strata 1 were in stems at the beginning of seed fill (R5) (Figure 2.19 – 2.14). This trend contrasts with the pattern of the whole plant, which showed that the N uptake was always greater in leaves. In the early planting date treatment, 20.1 – 33.6, 5.9 – 9.3, 35.2 – 60.0, and 9.3 – 18.1% of the N in strata 1 were partitioned to the leaves, petioles, stems, and pods, respectively. The remainder of the N in strata 1 was found in the fallen leaves and petioles (7.2 – 8.3%). In the mid-planting date, 0.7 – 17.2, 1.4 – 3.8, 42.3 – 70.6, and 7.3 – 32.6% of the N in strata 1 were partitioned to the leaves, petioles, stems, and pods, respectively. The remainder was found in the fallen leaves and petioles (4.2 – 18.1%). Finally, 7.0 – 16.6, 4.1 – 10.7, 51.1 – 65.6, and 9.4 – 17.1% of the N in strata 1 of the late-planted treatment was partitioned to the leaves, petioles, stems, and pods, respectively, while 5.7 – 14.2% was partitioned to the fallen leaves and petioles. After R5, N stored in vegetative organs starts remobilizing to the seed and senesced leaves. During seed fill (from R5 to maturity), 1.4 – 2.6, 0.6 – 0.8, and 1.7 – 2.0 g N m^{-2} (or 27.9 – 48.5, 30.5 – 32.5, and 56.2 – 57.2% of total vegetative N in strata 1) were remobilized to the seed for early, mid, and late-planting dates in 2020, respectively. In 2021, 1.4 – 2.0, 1.1 – 1.4, and 2.2 – 2.3 g N m^{-2} (52.8 – 59.1, 66.6 – 77.2, 76.2 – 82.3% of total vegetative N in strata 1) were remobilized to the seed for early, mid, and late-planting dates, respectively.

Strata 2. The N-partitioning patterns within strata 2 followed the pattern at the whole plant profile at the beginning of the seed fill (R5). Most of the N was partitioned to the leaves except for early planted Hoegemeyer LL2841 in 2021, which had a slightly lower N content in the leaves compared to stems. In general, 26.5 – 55.2, 7.3 – 9.8, 21.1 – 27.8, 11.3 – 30.5, and 0 – 7.8% of the total N within strata 2 was partitioned to the leaves, petioles, stems, pods, and fallen leaves and petioles, respectively, for the early planting date. At the mid-planting date, 34.5 – 44.9, 8.8 – 11.9, 22.4 – 31.1, 10.4 – 26.2, and 3.2 – 7.4% of the total N in strata 2 was partitioned to leaves, petioles, stems, pods, and fallen leaves and petioles, respectively. For the late-planting date, 42.1 – 53.2, 10.7 – 11.8, 18.9 – 23.6, and 15.0 – 19.4% of the N in strata 2 was partitioned to the leaves, petioles, stems, and pods, respectively. The remaining 1.0 – 3.6% of the N was found in fallen leaves and petioles. The contribution of total seed N from the vegetative N in strata 2 was greater than that of other strata. For instance, at R5, 4.1 – 4.7, 3.1 – 4.9, and 6.6 – 7.7 g N m^{-2} (69.9 – 70.5, 57.1 – 67.3, and 76.1 – 81.6% of the total vegetative N) in strata 2 were remobilized from leaves, petioles, stems, and pod walls to the seed for early, mid, and late-planting dates in 2020, respectively. In the 2021 growing season, 4.5 – 4.6, 5.5 – 6.1, and 7.1 – 8.4 g N m^{-2} of vegetative N was remobilized to seed between R5 and maturity (84.0 - 89.1, 78.0 – 84.4, and 81.8 – 82.6%) for early, mid, and late-planting dates, respectively.

Strata 3. In the middle of the whole plant profile, the proportion of N in the leaves was >45% at the beginning of the seed fill (R5). Some strata 3 leaves senesced in the mid-planting date treatment (2020) and for early planted Hoegemeyer LL2841 in 2021. Between 0.1 and 2.2% of the total N in strata 3 was lost to fallen leaves and petioles. In the early planting date, 50.5 – 59.4, 11.9 – 14.0, 16.0 – 21.0, and 7.5 – 20.5% of the N in strata 3 was partitioned to leaves, petioles, stems, and pods, respectively. For the mid-planting date, 46.6 – 60.7, 11.7 – 16.3, 18.1 – 21.5, and 4.8 – 18.2% of the total N in strata 3 was partitioned to leaves, petioles, stems, and pods, respectively. In the late planting date, 50.1 – 62.8, 11.8 – 14.8, 14.1 – 20.4, and 8.7 – 17.5% of the N was partitioned to leaves, petioles, stems, and pods, respectively. After this stage (R5), 3.9 – 4.4, 4.6, and 6.6 – 7.7 g N m^{-2} (53.9 – 64.1, 60.9 – 64.2, and 74.9 -79.0% of the vegetative N) in strata 3 was remobilized to the seed for early, mid, and late-planting dates in 2020, respectively. The 2021 growing season showed greater efficiency in remobilizing vegetative N from R5 to maturity (7.6 – 7.7, 6.6. – 7.4, and 5.4 – 6.3 g N m^{-2}, or 75.3 – 82.5, 65.3 – 75.4, and 80.5 – 81.5%) for early, mid, and late-planting dates, respectively.

Strata 4. The N-partitioning patterns within strata 4 followed a similar pattern to that of strata 3 at the beginning of seed fill (R5). Most of N was partitioned to leaves, with no fallen leaves or petioles. In the early planting date, N partitioned to the leaves, petioles, stems, and pods ranged from 54.8 – 62.3, 9.6 – 13.4, 14.1 – 15.8, and 9.2 – 19.7%, respectively. In the mid-planting date, 46.9 – 64.9, 10.7 – 15.6, 15.9 – 17.5, and 2.6 – 22.7% of the total N in strata 4 was partitioned to leaves, petioles, stems, and pods, respectively. For the late planting date, 49.3 – 65.1, 4.7 – 12.3, 11.3 – 23.6, and 16.8 – 23.1% of the N was partitioned to leaves, petioles, stems, and pods, respectively. During seed fill until maturity, 0.3 – 1.4, 2.8 – 3.1, and 1.4 – 2.4 g N m^{-2} (13.9 – 46.5, 60.4 – 62.1, and 51.6 – 66.7% of the vegetative N) was remobilized to seed for early, mid, and late-planting dates in 2020, respectively. In 2021, 2.8 – 3.3, 1.2 – 1.3, and 0.01 – 0.5 g N m^{-2} (65.1 – 67.5, 51.8 – 63.6, and 1.2 – 45.1% of the vegetative N) in strata 4 was remobilized to the seed for early, mid, and late-planting dates, respectively.

Strata 5. Since strata 5 was located at the top of the whole plant, the vegetative organs were still actively growing, as were the pods as generative organs. In all treatments at R5, 12.3 – 62.4, 4.3 – 22.8, 5.3 – 33.3, and 10.9 – 64.3% of the total N in strata 5 was partitioned to leaves, petioles, stems, and pods, respectively. The pattern of remobilization of vegetative N was different in strata 5. For the early planting date treatments in both years, late planted Pioneer P27A17X in 2020 and late planted Hoegemeyer LL2841 in 2021, there was no N remobilization from vegetative organs, as evidenced by increased N content in vegetative organs through maturity. On the other hand, 0.1 – 0.5 g N m^{-2} (12 - 48% of the vegetative N) in strata 5 was remobilized to seed in the mid-planting date in both years. As much as 25.3 and 17.3% of the total vegetative N in strata 5 was remobilized to seed in late planted Hoegemeyer LL2841 (2020) and Pioneer P27A17X (2021), respectively.

Source of final seed nitrogen

Nitrogen contribution to the final seed N was supported by the actual N uptake (both soil and fixed N) and remobilization from vegetative organs after R5. In the whole plant profile, the contribution of N remobilization generally was greater than from N uptake except for the early planting date treatment in 2020 (Figure 2.17). Final seed N in the early planting depended more on N uptake during seed fill, ranging from 55 – 59% in 2020. However, the majority final seed N was obtained from N remobilization from vegetative organs in 2021. Furthermore, the final seed N mainly depended on N remobilization from vegetative organs during seed fill in all other treatments.

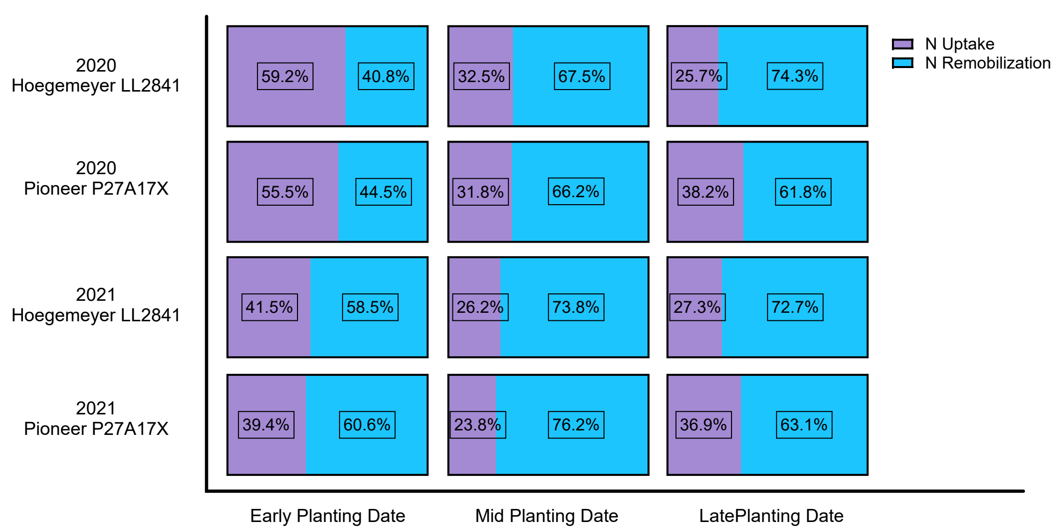

Figure 2.17. The contribution of nitrogen (N) from both N uptake and N remobilization from vegetative organs from the beginning of seed fill (R5) to the final seed N (R8) in the whole plant profile for each planting date treatment. Results are pooled among varieties.

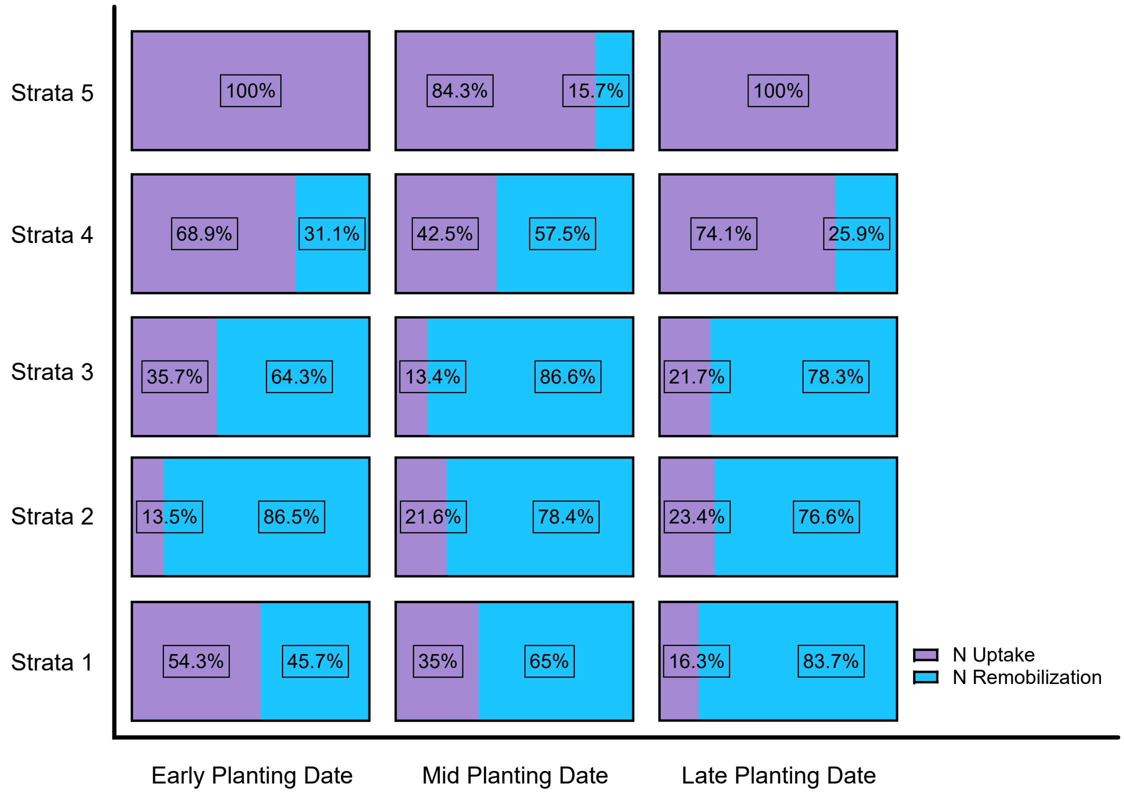

Figure 2.18. The contribution of nitrogen (N) from both N uptake and N remobilization from vegetative organs from the beginning of seed fill (R5) to final seed N (R8) within plant strata for each planting date treatment. Results are pooled among varieties.

Within the plant strata, the contribution of N uptake and remobilization from vegetative organs to final seed N showed a different pattern among planting dates (Figure 2.18). For instance, in the early planting, N uptake contributed more than 50% to the final seed N in the lower nodes (strata 1). N uptake also dominantly contributed to the final seed N in strata 4 and 5, ranging from 69% to 100%. However, in the middle strata (strata 2 and 3), N remobilization from vegetative organs contributed more than 64% to the final seed N within those strata. In the mid planting date treatment, majority of final seed N was obtained from N remobilization in strata 1, 2, 3, and 4, ranging from 58 to 87%. In comparison, N uptake contributed 84% to the final seed N in strata 5. In the late planting date treatment, N remobilization from vegetative organs dominated the contribution to final seed N in strata 1, 2, and 3, while N uptake after seed fill (R5) dominated the contribution to final seed N in strata 4 and 5.

Discussion

Dry matter accumulation

This study demonstrated that soybean dry matter (DM) accumulation is significantly influenced by planting date and variety, with notable differences observed between the 2020 and 2021 growing seasons. DM accumulation at R1 accounted for 6 - 21% of the total DM at maturity, depending on planting date treatment (Figure 2.1 – 2.6). Early planting resulted in longer periods of vegetative growth and slower initial DM accumulation rates (Kumudini et al., 2001; Gross et al., 2021). In contrast, mid and late-planting dates had shorter lag phases and higher initial DM accumulation rates (Park et al., 2015; Morris et al., 2021; Campos et al., 2024). Gaspar et al. (2017) showed that DM accumulation at R1 ranged from 11 – 14% regardless of yield level. In contrast, Bender et al. (2015) reported greater accumulation at R1 (20%). Even though DM accumulation differed among planting dates, the plant reached 50% DM accumulation at R3 for all treatments, earlier than reported by Hanway & Weber (1971), Farmaha et al. (2012), and Gaspar et al. (2017), who found 50% DM accumulation occurred at R4.

Since early planting date had a longer lag phase and growing period, it was in line with the lower peak DM accumulation rate compared to later planting dates. For instance, the peak rate for early, mid, and late-planting dates occurred before R3 and ranged from

19.5 – 22.4, 20.2 – 26.2, and 24.3 – 30.7 g m^{-2} day^{-1}, respectively (Figure 2.7 – 2.8). Gaspar et al. (2017) showed that the peak rate of DM accumulation was attained shortly after R3 with rates of 13.2, 14.9, and 17.1 g m^{-2} day^{-1} for low, mid, and high yield levels, respectively. Bender et al. (2015) reported a peak DM accumulation rate of 16.2 g m^{-2} day^{-1} at R4. In contrast, Matree and Toyota (2017) reported a peak DM accumulation of 26.2 g m^{-2} day^{-1} for the Hatsusayaka cultivar in two different densities. After the peak DM accumulation period, the rate steadily slowed to 3.0 to 5.4 g m^{-2} day^{-1} for early planting date, 0.8 to 2.7 g m^{-2} day^{-1} for mid planting date, and 1.1 to 3.3 g m^{-2} day^{-1} for late planting date at R7 (Figure 16 & 17). This was consistent with that reported by Gaspar et al. (2017). At the end of the growing season, total DM accumulated ranged from 976 – 1095, 772 – 922, and 870 – 1054 g m^{-2} for early, mid, and late planting dates, respectively (Figure 2.1 – 2.6). Gaspar et al. (2017) reported that high-yield varieties resulted in 1036 g m^{-2} while the lower-yield level resulted in 741 g m^{-2}. Our study found a positive correlation between total DM and seed yield (R8) with an $r = 0.81$ ($P < 0.0001$). However, this correlation was not due to the length of the lag phase of the early season, where a short lag phase in the early season did not always result in a greater total DM and higher yield, as Gaspar et al. (2017) stated that greater total DM at R8 was consistently detected with higher yields likely due to a shorter duration in the lag phase of the early season.

Dry matter (DM) accumulation patterns exhibit notable differences among the various plant strata, significantly affecting overall DM accumulation in the whole plant profile. The stratified dry matter (DM) accumulation patterns observed in soybean plants suggest a strategic resource allocation strategy to optimize biomass production and yield potential. This study observed that the lower strata (1 and 2) primarily accumulated DM early in the growing season, while other strata (3, 4, and 5) primarily accumulated after flowering (R1). This allocation strategy ensures a strong foundation for the plant's development. As the plant progresses through growth stages, there was a shift towards increased DM accumulation in the upper strata. This transition in resource allocation signifies a coordinated effort within the plant to maximize biomass production and yield potential by strategically distributing resources across different growth stages and plant parts (Taylor et al., 2005; Zobiole et al., 2012; Dhakal, 2024).

Dry matter partitioning

The study revealed significant variation in dry matter (DM) partitioning across planting date and soybean variety. New growth ceased to be partitioned to leaves and petioles by R5, except for Hoegemeyer LL2841 in the mid (2021) and late planting dates (2020 & 2021). After R5, DM accumulation was directed toward seeds and pods and the apparent remobilization of carbohydrates from vegetative organs (Figure 2.1 – 2.6) (Hanway & Weber, 1971; Bender et al., 2015). However, the continued partitioning of DM to stems and pods (and leaves and petioles for Hoegemeyer mentioned above) until mid-R6 for all treatments resulted in more vegetative DM. This possibly contributed to more pods per square meter, which is considered an important yield component (Gaspar et al., 2017).

Within strata, partitioning of new growth to leaves and petioles ceased at different growth stages. For instance, the lower strata (strata 1) ceased new leaf and petiole growth no later than R3 in all treatments, no later than R5 for strata 2, and no later than R6 for strata 3, 4, and 5. However, continued partitioning of DM to stems continued until R6 in all treatments. Remobilization of leaf and petiole DM from strata 1 to other vegetative organs (stems) occurred prior to the initiation of seed fill, while stems supported high vegetative DM remobilization at the end of the growing season.

Nitrogen uptake

The whole plant total N uptake was 31.9 – 36.7, 25.7 – 28.8, and 26.1 – 37.4 g N m^{-2} for early, mid, and late planting dates, respectively. These numbers are within the range of total N uptake across widely different yield levels reported by Gaspar et al. (2017) (22.9 – 34.8 g N m^{-2}). Bender et al. (2015) reported slightly lower N uptake, ranging from 25.9 – 31.8 g N m^{-2}. Moreover, for a wide range of reported soybean yields over a 50-year period, Salvagiotti et al. (2008) reported total N uptake averaging 21.9 g N m^{-2}, with a range of 4.4 – 48.5 g N m^{-2}.

Nitrogen uptake rate by R1 accounted for < 14% of the total season-long N uptake, except for both varieties in mid planting date in 2020 and late planting date in 2021 (Figure 2.15 & 2.16). This is consistent with Gaspar et al. (2017), who reported 14, 13, and 12% of total N uptake at R1 for low, average, and high yield levels, respectively. Early planting treatments reached peak nitrogen uptake later compared to mid and late planting treatments. Peak nitrogen uptake rate ranged from 0.55 – 0.82 g N m^{-2} day^{-1} for all treatments, higher than that reported in Gaspar et al. (2017) (0.36 to 0.43 g N m^{-2} day^{-1} and Bender et al. (2015) (0.43 to 0.48 g N m^{-2} day^{-1}).

The peak nitrogen uptake rate was greatest in strata 2 and 3, which significantly contribute to the overall nitrogen distribution within the plant. This critical process, particularly translocating nitrogenous compounds such as amino acids to developing embryos, is pivotal in determining seed yield and quality (Bennett and Spanswick, 1983). The greater contribution of the middle and upper canopy strata to grain yield and N content is attributed to the higher density of pods in these strata (Schwerz et al., 2019).

Nitrogen partitioning

In the beginning of seed fill (R5), most of the N was partitioned to leaves (up to 52% of the whole plant N). The rest of the N was located in petioles, stems, and pods. From R5, nitrogen remobilized from vegetative organs to the seed rapidly until maturity (R8). In the early and mid-planting date treatments (2020), only 50 to 59% of the vegetative nitrogen was remobilized to seed. Hanway & Weber (1971) reported this number to be ~55%. However, the late planting treatment in 2020 and all treatments in 2021 remobilized 67.5 to 79.4% of vegetative N to seed. These values are similar to the fraction of vegetative N remobilized to seed by Gaspar et al. (2017) and Vasilas et al. (1995).

Seed nitrogen from accumulated uptake (soil and fixed N) after R5 was greatest in the early planting date treatment in 2020, accounting for 59.3 and 55.5% of the total seed N for Hoegemeyer LL2841 and Pioneer P27A17X, respectively. However, N accumulated from continued uptake only accounted for 39.5 – 41.6, 23.7 – 32.6, and 25.8 – 38.2% for early, mid, and late-planting dates, respectively. Remobilization of vegetative N is important in meeting seed N demand in soybean. This agrees with

Sinclair and de Wit (1976), who stated that greater vegetative N uptake and storage before R5.5 for successive remobilization to the seed was necessary to increase soybean yields significantly. Nonetheless, it counters the result from Gaspar et al. (2017) and Loberg et al. (1984), who stated that greater reliance on continued uptake as an alternative to vegetative N remobilization after R5 was associated with higher yields.

This study showed that the proportion of total nitrogen uptake varies among plant strata at the R8 stage. Our results show that, at the whole plant level, remobilization of vegetative N is the primary driver of the total seed N content at maturity, with support from continued uptake. Strata 2 and 3 contribute >47% of the total soybean seed N content at maturity.

Harvest index

The harvest index (HI) is a crucial parameter that reflects the efficiency of a plant in partitioning assimilated biomass to the harvested seeds. This study showed variation in HI across planting dates and soybean varieties and a positive correlation with seed yield ($r = 0.67$; $P < 0.0001$). A high HI indicates a plant's ability to allocate more of its total biomass to seed production, directly influencing final yield. Results were consistent with previous research demonstrating a positive correlation between HI and seed yield (Foulkes et al., 2011; Sinclair & Vadez, 2012). Egli (2004) reported that improvements in HI were often associated with breeding efforts to enhance yield potential in various crop species, including soybeans.

Nitrogen harvest index (NHI)

The NHI measures the proportion of total plant N allocated to seeds, reflecting nitrogen use efficiency for grain production. This study found significant variability in NHI across planting dates and varieties, with late planting dates generally showing higher NHI values. A positive correlation was observed between NHI and seed yield ($r = 0.49$; $P = 0.0004$). Higher NHI values indicate a plant's effective nitrogen remobilization from vegetative organs to reproductive structures (seeds). Salvagiotti et al. (2008) and Ciampitti & Vyn (2013) highlighted the importance of efficient nitrogen remobilization in maximizing seed yield.

However, it is essential to consider that environmental conditions, management practices, and genetic factors influence the relationship between HI, NHI, and yield. While this study demonstrates a clear correlation, variation in environmental conditions and management practices can affect these indices and their relationship with yield. Studies by Boote et al. (1998) and Bender et al. (2015) have shown that factors such as water availability, soil fertility, and pest management can influence HI and NHI, thereby affecting yield outcomes.

Conclusion

Our results indicate that the accumulation of DM and N in the whole plant is minimal until approximately R1, then increases rapidly to the peak near R3 for all treatments. After R5, vegetative organs (leaves and petioles) generally began remobilizing DM and N to the seed. In contrast, stems began DM and N remobilization at R6, possibly supporting greater DM remobilization to the seed. Within strata, peak accumulation of DM and N shifted from R1 to R7 from the bottom of the plant (strata 1) to the top (strata 5). Moreover, vegetative organs varied in their time of maximum DM or N accumulation, and when remobilization of DM or N to seed was initiated. Our results showed that at the whole plant level, seed N accumulation primarily relied on remobilization from vegetative organs rather than on continued uptake. Strata 2 and 3 contributed most of the remobilized N to seed.

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