Article

Contribution of Nitrogen Uptake and Retranslocation during Reproductive Growth to the Nitrogen Efficiency of Winter Oilseed-Rape Cultivars (*Brassica napus* L.) Differing in Leaf Senescence

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Abstract: Genotypic variation in N efficiency defined as high grain yield under limited nitrogen (N) supply of winter oilseed-rape line-cultivars has been predominantly attributed to N uptake efficiency (NUPT) through maintained N uptake during reproductive growth related to functional stay-green. For investigating the role of stay-green, N retranslocation and N uptake during the reproductive phase for grain yield formation, two line cultivars differing in N starvation-induced leaf senescence were grown in a field experiment without mineral N (N0) and with 160 kg N· ha^{-1} (N160). Through frequent harvests from full flowering until maturity N uptake, N utilization and apparent N remobilization from vegetative plant parts to the pods could be calculated. NUPT proved being more important than N utilization efficiency (NUE) for grain yield formation under N-limiting (N0) conditions. For cultivar differences in N efficiency, particularly N uptake during flowering (NUPT) and biomass allocation efficiency (HI) to the grains, were decisive. Both crop traits were related to delayed senescence of the older leaves. Remobilization of N particularly from stems and leaves was more important for pod N accumulation than N uptake after full flowering. Pod walls (high N concentrations) and stems (high biomass) mainly contributed to the crop-residue N at maturity. Decreasing the crop-inherent high N budget surplus of winter oilseed-rape requires increasing the low N remobilization efficiency particularly of pod-wall N to the grains. Addressing this conclusion, multi-year and -location field experiments with an extended range of cultivars including hybrids are desirable.

Keywords: *Brassica napus*; line cultivars; genotypic differences; nitrogen efficiency; nitrogen uptake; nitrogen utilization; nitrogen retranslocation; stay-green

1. Introduction

Winter oilseed-rape (*Brassica napus* L.) is the most important oil crop in northern Europe. The oil is used for human consumption (edible oil) or industrial purposes (lubricant and biodiesel) [1].

In most cropping systems, nitrogen (N) availability is one of the major factors determining crop growth and thus yield formation. For optimum grain yield, winter oilseed-rape requires about 200 kg N· ha^{-1} [2]. Under best agronomic practices, a grain yield of 4.5 t· ha^{-1} causes an N budget surplus of around 60 kg N· ha^{-1} for which an incomplete depletion of plant available soil-N by the crop and an incomplete retranslocation of N from vegetative plans parts into the grains are responsible [3,4]. Thus, a substantial part of the N required for optimal crop development is

not removed from the field with the harvested grains. For economic and ecological reasons, the high crop-specific N balance surplus has to be reduced, but without reducing the current yields. A promising approach is the breeding and cultivation of N-efficient (high yield under N limitation) cultivars [5] that allow decreasing the N application necessary to achieve optimum yields. Nitrogen efficiency is a complex crop trait, which is based on two pillars: (I) the effectiveness of a cultivar in absorbing nutrients from the soil (N uptake efficiency (NUPT)) and/or (II) the efficiency with which the N is utilized to produce yield (N utilization efficiency (NUE)) [6]. The relative contribution of either efficiency trait to N efficiency depends not only on the crop species [7,8] but also on the severity of the N deficiency stress, as could be shown for winter oilseed-rape [9–11]. Genotypic variation in N efficiency in winter oilseed-rape has been mainly attributed to NUPT particularly after transition from the vegetative to the reproductive developmental period during flowering [9,10,12].

Insufficient N supply induces and accelerates senescence [13], which causes enhanced crop maturation and thus reduces yields [14]. A characteristic of N-efficient cultivars with prolonged N uptake into the reproductive growth phase under N-limiting conditions is a functional stay-green phenotype, expressed as delayed senescence of the older leaves, accompanied by maintenance of the photosynthetic capacity [15]. Despite the fact that it is currently not clear whether the stay-green phenotype of N-efficient cultivars is the cause or the consequence of maintained root growth [16], it is assumed that the prolonged assimilate supply to the roots enhances the N uptake into the reproductive phase because of extending the leaf-photosynthesis duration.

However, for yield formation and low amounts of N remaining in the crop residues plant senescence finally, is a prerequisite. A key function of senescence is the remobilization of particularly N from the vegetative plant parts to reproductive organs (particularly the grains) [17]. In winter oilseed-rape, more than 70% of pod N is derived from N remobilization [18,19]. Exploiting genotypic variation in N remobilization efficiency could contribute to enhance N efficiency of oilseed rape [20].

The aim of the study was to investigate under field conditions the role of stay-green and the importance of the amount and the timing of N retranslocation and N uptake during the reproductive phase for genotypic variation in N efficiency of two winter oilseed-rape line-cultivars differing in N starvation-induced leaf senescence [21,22].

2. Results

2.1. Leaf Senescence

The commercial line-cultivars Apex and Capitol, which had been shown to differ in N efficiency and functional stay green [11,15,21,22], were included into the present field study. As reliable parameter of leaf senescence under field conditions [11,15], the number of green leaves remaining on the plant's main stem was counted from flowering (BBCH65) until near maturity. During reproductive growth, the percentage of green leaves decreased substantially for both cultivars (cvs.) and N supplies (Figure 1). The decline was significantly faster at N0 than at high N supply (significant N × DAFF interaction). At N0 but not at N160 the number of green leaves was consistently higher for cultivar (cv.) Apex than for cv. Capitol from DAFF 10 onward. Thus under N limitation the stay-green phenotype of cv. Apex could be confirmed.
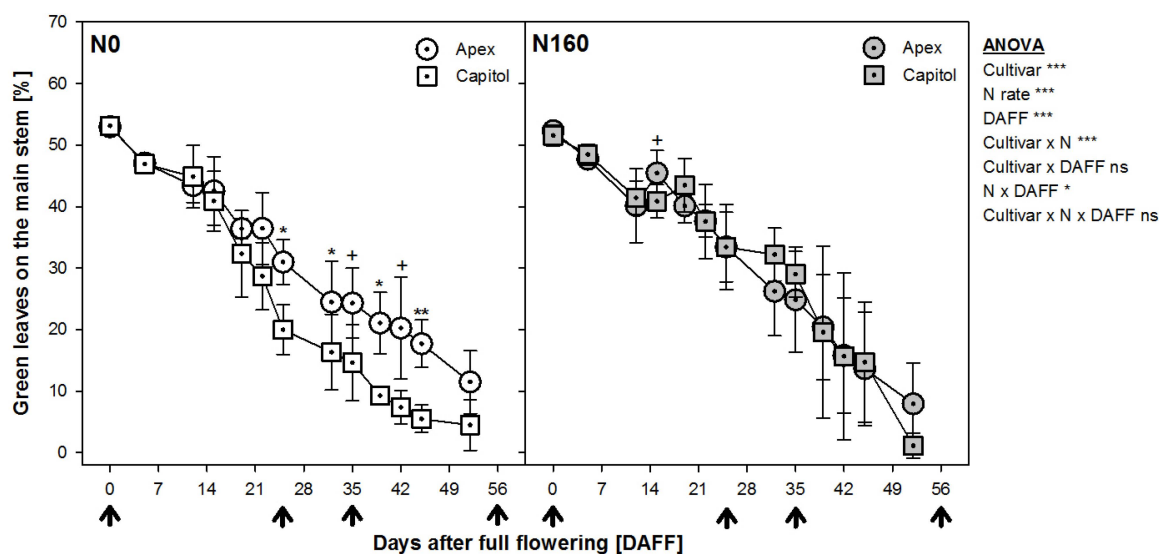
Figure 1. Remaining green leaves on the main stem of the winter oilseed-rape line-cultivars Apex and Capitol from full flowering until near maturity as affected by the N fertilization rate (N0 and N160). The arrows indicate the developmental stages BBCH65, BBCH69, BBCH79, and BBCH89, respectively. ANOVA: ******* and ***** indicate significant differences at $p < 0.05$ and $p < 0.001$, respectively. ns = non-significant. At individual DAFF: **+**, *****, and ****** indicate significant differences between the cultivars within the N fertilization rates at $p < 0.10$, $p < 0.05$, and $p < 0.01$, respectively. The error bars (visible only when greater than the symbols) represent the standard deviations of the means ($n = 3$–4).

2.2. Grain Yield and Straw Dry Matter

At maturity (BBCH89) grain yields of cvs. Apex and Capitol were significantly affected by the N fertilization rate (Figure 2A). Under high N supply (N160), grain yields of both cultivars did not differ significantly. Without N fertilization (N0) grain yields were substantially lower and cv. Apex reached a significantly higher grain yield compared to cv. Capitol leading to a significant cultivar × N interaction. Generally, the grain yields were low. This was mainly due to the low plant density, because the grain yields per plant under high N supply (N160) were 8 to 10 g per plant for all cultivars which could have allowed grain yields of 5 to 6 t·ha^{-1} at the target plant density of 60 plants·m^{-2}. In conclusion, the previously reported higher N efficiency of cv. Apex compared to cv. Capitol could be confirmed. For both cultivars straw dry matter production (shoot + pod walls) at maturity (BBCH89) was significantly increased by N fertilization (Figure 2B). Under high N supply (N160) both cultivars reached a similar straw dry weight (7.9 to 8.1 t·ha^{-1}) and no significant differences occurred between the cultivars. However, under N limitation (N0) the N-efficient cv. Apex reached a significantly higher straw dry weight than cv. Capitol explaining the significant cultivar × N interaction.
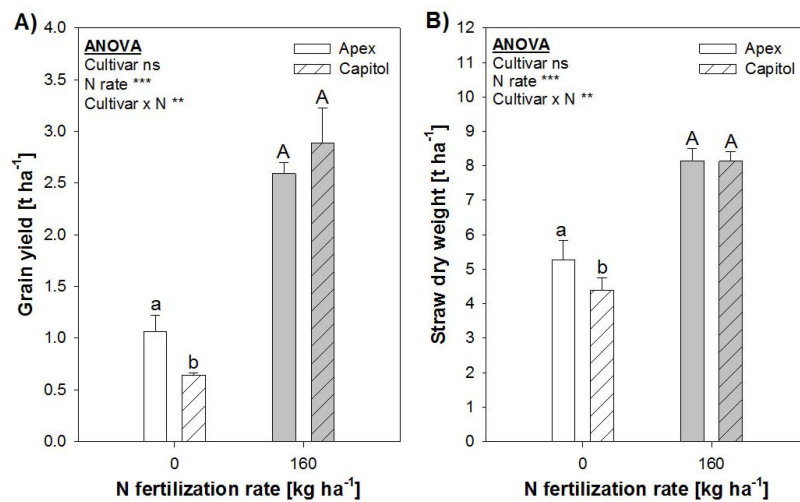
Figure 2. (**A**) Grain yield and (**B**) straw dry weight at maturity of the winter oilseed-rape line-cultivars Apex and Capitol as affected by the N fertilization rate (N0 and N160). Different lower case and upper case letters on the top of the columns indicate differences between the cultivars within the N fertilizer rates at $p < 0.05$. ANOVA: ** and *** indicate significant differences at $p < 0.01$ and $p < 0.001$, respectively. ns = non-significant. The error bars represent the standard deviations of the means ($n = 3$–4).

2.3. Biomass Distribution Efficiency

The separate determination of straw and grain dry weights allowed the calculation of the biomass-distribution efficiency for grain-yield formation, for which the harvest index (HI) is a measure. In general N fertilization significantly increased the HI at maturity (BBCH89) (Figure 3). Under high N supply (N160), the HI was similar and did not differ between both cultivars. Under N limitation (N0), the HI of cv. Apex was significantly higher compared to cv. Capitol. The significant cultivar × N interaction was based on the higher HI of cv. Apex under N limitation (N0).
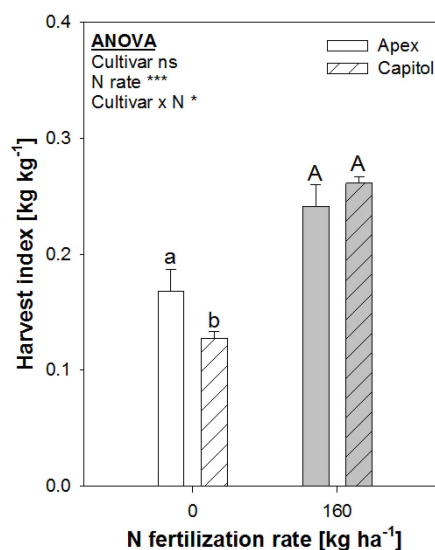

Figure 3. Harvest index at maturity of the winter oilseed-rape line-cultivars Apex and Capitol as affected by the N fertilization rate (N0 and N160). Different lower case and upper case letters on top of the columns indicate differences between the cultivars within the N fertilizer rates at $p < 0.05$. ANOVA: * and *** indicate significant differences at $p < 0.05$ and $p < 0.001$, respectively. ns = non-significant. The error bars represent the standard deviations of the means ($n = 3$–4).

2.4. Shoot Nitrogen Uptake

At high N supply (N160) N uptake by shoots reached 120 to 148 kg N·ha^{-1} at flowering (BBCH65) and increased up to DAFF 42 to 131 to 177 kg N·ha^{-1} (Figure 4). At limiting N supply (N0) the N uptake until flowering was about two times lower (42 to 45 kg N ha^{-1}) and increased only until DAFF 57 to 67 to 79 kg N·ha^{-1}. At N0 but not at N160 cv. Capitol showed a consistent and mostly significantly lower N uptake from DAFF 14 until maturity than cv. Apex. The magnitude of post-flowering N uptake at N0 was significantly higher for cv. Apex (31 kg N·ha^{-1}) than for cv. Capitol (10 kg N·ha^{-1}).
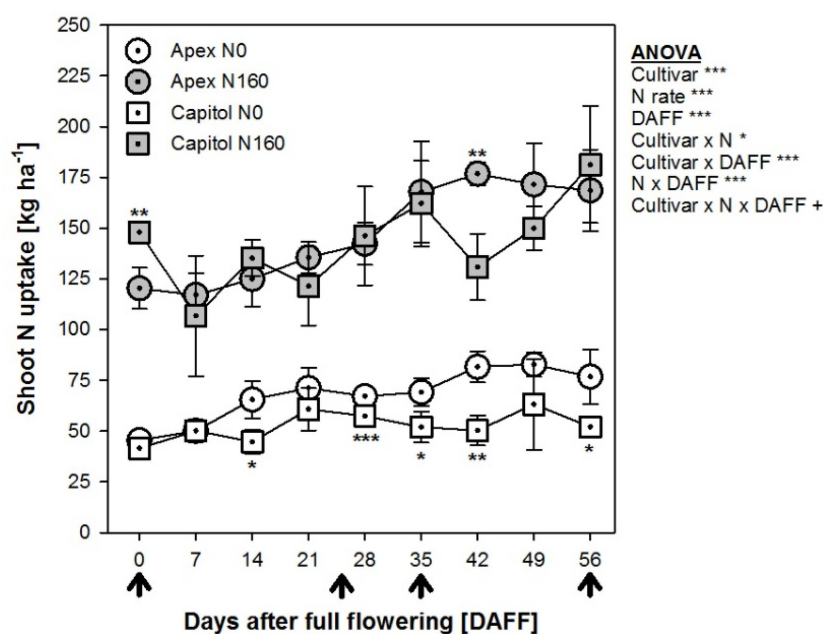

Figure 4. Shoot N uptake from full flowering until maturity of the winter oilseed-rape line-cultivars Apex and Capitol as affected by N fertilization rate (N0 and N160). The arrows indicate the developmental stages BBCH65, BBCH69, BBCH79, and BBCH89, respectively. ANOVA: +, *, and *** indicate significant differences at $p < 0.10$, $p < 0.05$, and $p < 0.001$, respectively. ns = non-significant. Individual DAFF: *, **, *** indicate significant differences between the cultivars within the N fertilization rates at $p < 0.05$ and $p < 0.01$ and $p < 0.001$, respectively. The error bars (visible only when greater than the symbols) represent the standard deviations of the means (n = 3–4).

2.5. Nitrogen Accumulation in Pods from Flowering until Maturity

After transition to the reproductive phase the developing pods are the main accumulation sites for N. From full flowering (BCCH65) until maturity (BBCH89) the N contents of the pods increased under both N supplies with a significantly higher increase under high N supply (N160) (Figure 5). Under high N fertilization (N160) the accumulation generally continued up to about DAFF 42. Without N fertilization (N0) N accumulation continued only beyond DAFF 28 in the pods of cv. Apex and thus significantly longer compared to cv. Capitol (highly significant Cultivar × N × DAFF interaction). Consequently, the N accumulation in the pods was significantly greater in cv. Apex compared to cv. Capitol at limiting but not at high N supply (significant Cultivar × N × DAFF interaction).

The separation of the pods into pod-walls and grains from BBCH79, when all pods had reached the cultivar-specific size, until maturity (BBCH89) revealed that N accumulation in the grains continued up to DAFF 49 independently of cultivar and N fertilization rate, but at a much higher rate at high compared to limited N supply (Figure 6A). However, cv. Capitol continued to accumulate N in the grains until maturity at N160. In agreement with the lower grain yield at N0 (Figure 2A), cv. Capitol accumulated significantly less N in the grains than cv. Apex. The increase in N contents of the grains during reproductive growth were exclusively due to the increase in grain mass since the grain-N

concentrations did not change (Figure 6A). The grain-N concentrations of cvs. Apex and Capitol did not respond to the N fertilization rate and did not differ.
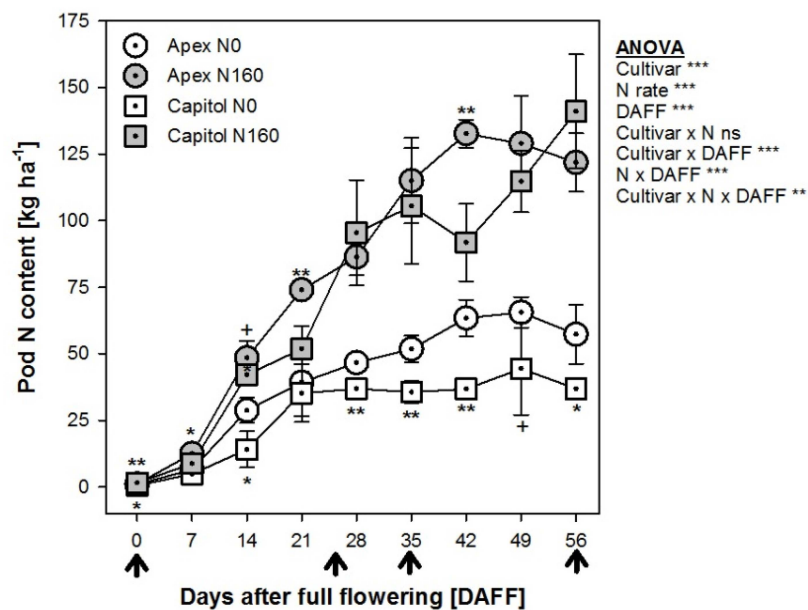

Figure 5. Nitrogen accumulation in the pods from full flowering until maturity of the winter oilseed-rape line-cultivars Apex and Capitol as affected by N fertilization rate (N0 and N160). The arrows indicate the developmental stages BBCH65, BBCH69, BBCH79, and BBCH89, respectively. ANOVA: ** and *** indicate significant differences at $p < 0.01$ and $p < 0.001$, respectively. ns = non-significant. Individual DAFF: +, *, and ** indicate significant differences between the cultivars within the N fertilization rates at $p < 0.10$, $p < 0.05$, and $p < 0.01$, respectively. The error bars (visible only when greater than the symbols) represent the standard deviations of the means (n = 3–4).

During the same period (DAFF 35 to 49) of increasing grain N contents, the N contents of the pod walls decreased (Figure 6B). The increase in N accumulation in the grains and the decrease of the N contents of the pod walls was particularly marked at high N supply (N160), whereas the pod-N contents did not markedly change at N0. Still, at high N supply, the amounts of N remaining in the pod walls at maturity (DAFF 56) were significantly greater (42 to 44 kg N·ha^{-1}) compared to N0 (17 to 23 kg N·ha^{-1}) with no significant differences between both cultivars. The N concentrations of the pod walls were generally about two times lower than of the grains (compare Figure 6A,B). They were significantly higher at the high N supply in both cultivars. The decline in pod-wall N contents was based on decreased pod-wall N concentrations. Although the N concentrations of the pod walls also decreased from DAFF 35 until maturity (DAFF 56) at N0, the pod-wall N contents did not markedly change.
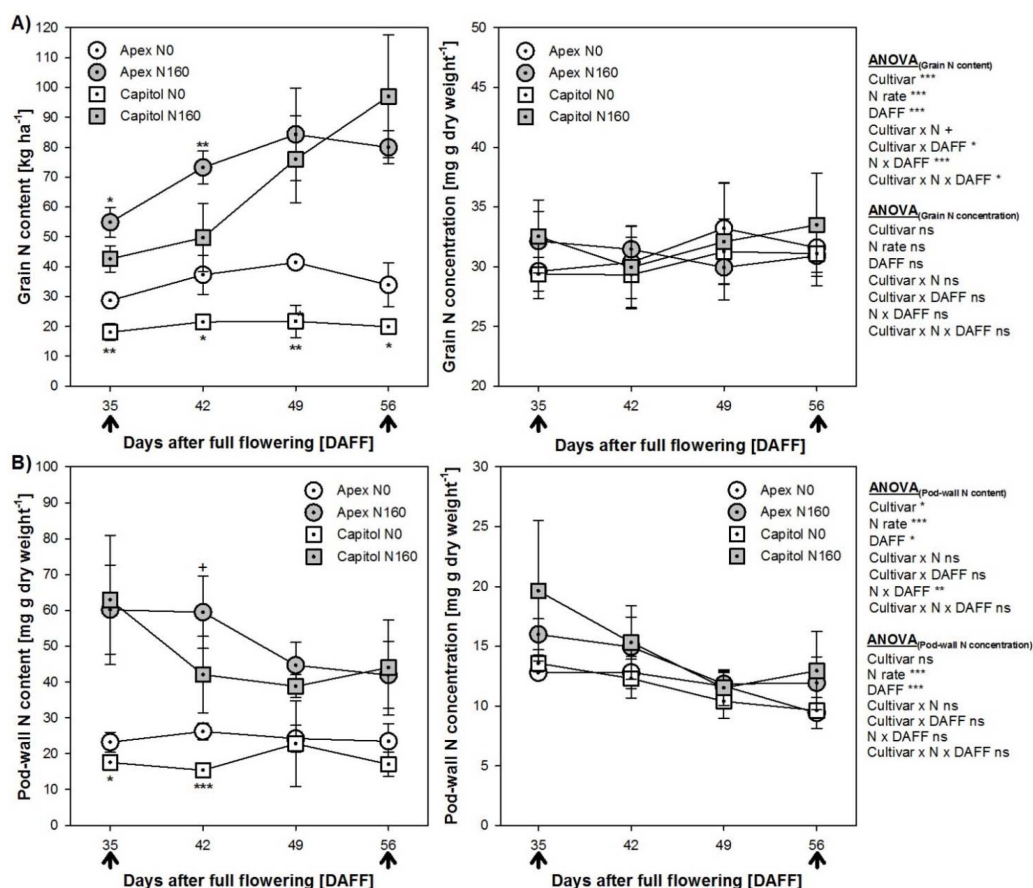
Figure 6. Nitrogen contents (**left**) and concentrations (**right**) of the (**A**) grain and (**B**) pod wall from all pods having reached the cultivar-specific size until maturity of the winter oilseed-rape line-cultivars Apex and Capitol as affected by N fertilization rate (N0 and N160). The arrows indicate the developmental stages BBCH79 and BBCH89. ANOVA: +, *, **, and *** indicate significant differences at $p < 0.10$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. ns = non-significant. Individual DAFF: +, *, **, and *** indicate significant differences between the cultivars within the N fertilization rates at $p < 0.10$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. The error bars (visible only when greater than the symbols) represent the standard deviations of the means ($n = 3$–4).

2.6. Apparent N Uptake and Retranslocation to the Pods

The increase in pod N content is based on two processes: N uptake during reproductive growth and direct transport to the pods, and retranslocation from vegetative plant organs of N taken up during vegetative growth. Apparent remobilization of N in vegetative plant organs (roots, stems, and leaves) for transport to the pods can be quantified through the decrease in N contents of these organs. In the taproot, the N contents were significantly affected by the level of N fertilization and higher at N160 compared to N0 (Figure 7A). The taproot N content decreased only slightly but significantly from full flowering until maturity mainly at high N supply (N160) for both cultivars (significant N × DAFF interaction). Particularly at N160, cv. Apex generally had higher taproot N contents. The decrease in N contents after full flowering of the taproots and the differences between the N fertilization rates can be mostly explained by the differences in taproot N concentrations (Figure 7A). However, the mostly higher N contents of the taproots of the late-senescing cv. Apex are not related to higher N concentrations but rather to a higher taproot biomass.

In contrast to the taproot, the stem proved to be a major intermediate storage organ for N at high N fertilization rate (N160) (Figure 7B). After full flowering, this N pool was gradually depleted more rapidly at N0 compared to N160 (significant N × DAFF interaction). At maturity the amount of N

remaining in the stems was clearly higher at N160. The decrease in N contents of the stems after full flowering and the difference between the N fertilization rates can be fully explained by the stem N concentrations (Figure 7B). Differences between the cultivars were negligible.

The amount of N in intact leaves at full flowering was lower than in stems (Figure 7C). As for stems the N contents of the intact leaves decreased more rapidly under high compared to limiting N supply for both cultivars. However, the decline was less rapid and less complete in cv. Apex particularly at high N supply (N160). The N amounts remaining in intact leaves reached much lower levels than in the stems. This can be attributed to the shedding of nearly all leaves until maturity which is supported by an increasing N content of shed leaves (Figure 7D). The higher N content of intact leaves at high N supply in cv. Apex can be explained by a delayed leaf shedding reflected by a slower increase in N content of shed leaves. At low N supply the cultivars did not differ in N contents in stems and leaves. Among all vegetative plant organs the intact leaves had the highest N concentrations (Figure 7C) clearly higher at N160 compared to N0. With development the N concentrations decreased, but less than the N contents supporting the major role of leaf shedding for the decline in leaf N contents. Close to maturity the differences between the N fertilization rates disappeared and the N concentrations of the intact leaves even increased again which can be explained by the decreasing leaf age of the final remaining leaves on the inflorescences. Cultivars Apex and Capitol differed with generally slightly higher leaf-N concentrations of cv. Capitol clearly suggesting that the higher N contents of cv. Apex were due to delayed leaf shedding. The N concentrations in the shed leaves decreased up to maturity to very low values in N-deficient but less in N-sufficient plants (highly significant N × DAFF interaction). Cultivars Apex and Capitol differed significantly but this was quantitatively insignificant.

The monitoring of the change in N contents of the different plant organs from full flowering (BBCH65) to maturity (BBCH89) allowed to separate the pod-N accumulation into N uptake directly allocated to the pods (apparent N uptake) and N retranslocated from vegetative plant organs (apparent N retranslocation) (Table 1). Under N-limiting conditions (N0), the cvs. Apex and Capitol showed a similar apparent N retranslocation to the pods. N fertilization (N 160) significantly increased the apparent N retranslocation to the pods in cv. Capitol (115 kg N·ha^{-1}) more than in cv. Apex (75 kg N·ha^{-1}).

Table 1. Apparent N retranslocation and apparent N uptake directly allocated to the pods from full flowering to maturity of the winter oilseed-rape line-cultivars Apex and Capitol as affected by the N fertilization rate (N0 and N160). ANOVA: +, *, **, and *** indicate significant differences at $p < 0.10$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. ns = non-significant. Different lower case and upper case letters indicate significant cultivar differences within the cultivars at N0 and N160, respectively, at $p < 0.05$. SD = Standard deviations of the means ($n = 3$–4).

Cultivar	Apparent N Retranslocation				Apparent N Uptake			
	Mean		**SD**		**Mean**		**SD**	
	(kg· ha^{-1})							
N0								
Apex	23	±	8	a	34	±	19	a
Capitol	28	±	1	a	9	±	3	a
N160								
Apex	75	±	20	B	47	±	25	A
Capitol	115	±	9	A	26	±	29	A
ANOVA								
Cultivar	**				+			
N rate	***				ns			
Cultivar × N	*				ns			
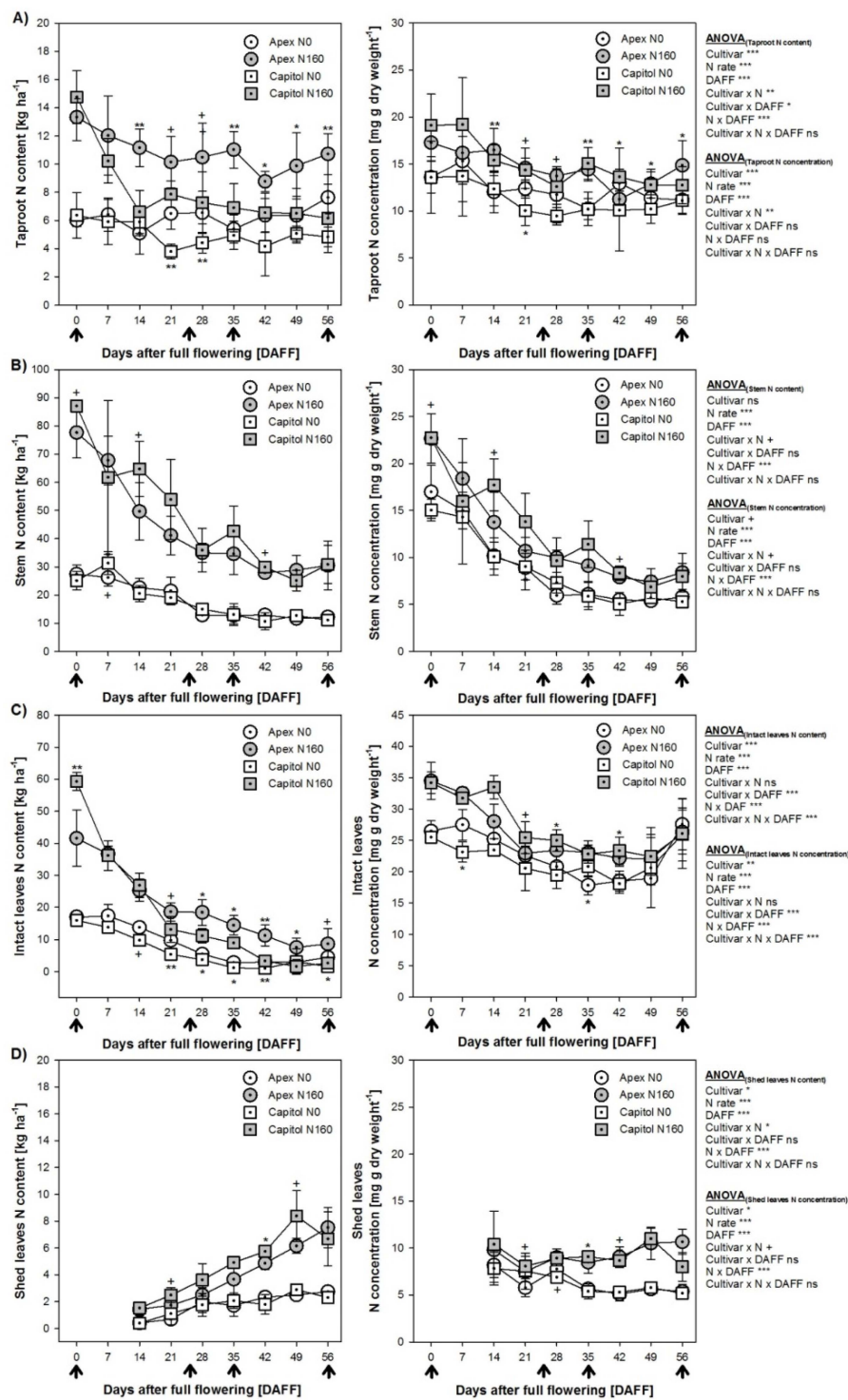
Figure 7. Nitrogen content (**left**) and concentration (**right**) of the (**A**) taproot, (**B**) stem; (**C**) intact leaves and (**D**) shed leaves from full flowering until maturity of the winter oilseed-rape line-cultivars Apex and Capitol as affected by N fertilization rate (N0 and N160). The arrows indicate the developmental stages BBCH79 and BBCH89. ANOVA: +, *, **, and *** indicate significant differences at $p < 0.10$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. ns = non-significant. Individual DAFF: +, *, **, and *** indicate significant differences between the cultivars within the N fertilization rates at $p < 0.10$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. The error bars (visible only when greater than the symbols) represent the standard deviations of the means (n = 3–4).

The apparent N uptake by the pods was calculated as the difference between the pod-N content and the apparent N retranslocation. The overall statistical analysis revealed that apparent N uptake was not or only little ($p < 0.10$) affected by the level of N fertilization. At maturity at N0, cv. Apex (34 kg N·ha^{-1}) showed a nearly four-times higher apparent N uptake compared to its counterpart cv. Capitol (9 kg N·ha^{-1}), but owing to the high variation the difference in apparent N uptake between the cvs. Apex and Capitol was only significant at $p < 0.10$.

2.7. Nitrogen Utilization Efficiency and N Harvest Index

The N utilization efficiency (NUE) reflects the efficiency with which N is utilized to produce yield. For the four cultivars the NUE ranged from 31 to 32 kg·kg^{-1} without significant cultivar and N application effects (Figure 8A).

The nitrogen harvest index (NHI) is a measure for the efficiency of the allocation of N to the grains (uptake and retranslocation). The high N fertilization rate (N160) generally increased NHI (Figure 8B). Cultivar Apex showed a higher NHI than cv. Capitol at N0 but a lower NHI at N160 (significant cultivar × N interaction).
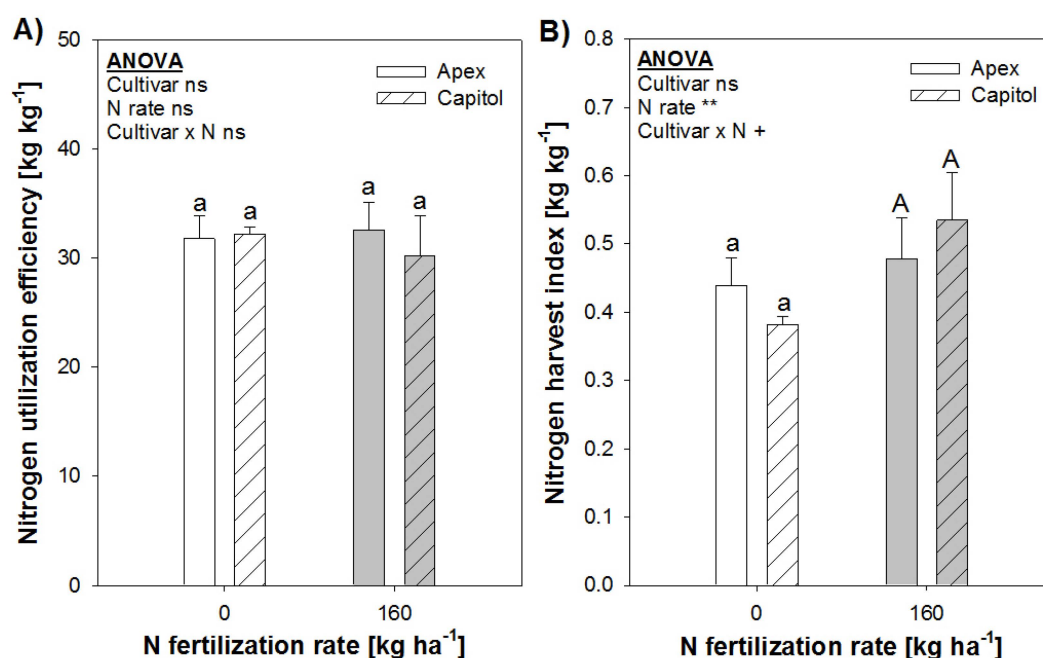

Figure 8. (**A**) Nitrogen utilization efficiency and (**B**) N harvest index at maturity of the winter oilseed-rape line-cultivars Apex and Capitol as affected by the N fertilization rate (N0 and N160). Different lower case and upper case letters on the top of the columns indicate differences between the cultivars within the N fertilizer rates at $p < 0.05$. ANOVA: + and ** indicate significant differences at $p < 0.10$ and $p < 0.01$, respectively. ns = non-significant. The error bars represent the standard deviations of the means ($n = 3$–4).

2.8. Nitrogen Remaining in Crop Residues

The NHI suggests that at maturity (BBCH89) only approximately 50% of the total N in the aboveground plant-parts and the taproot was removed from the field with the grains (Figure 8). Without N fertilization (N0) the remaining N in the crop residues (taproot, stem, intact leaves, shed leaves and pod-wall) summed up to 36.9 kg N·ha^{-1} for cv. Capitol and 50.6 kg N·ha^{-1} for cv. Apex (Figure 9). At high N fertilization rate (N160), the remaining N in the crop residues was about two times higher with 90.5 kg N·ha^{-1} for cv. Capitol and 99.4 kg N·ha^{-1} for cv. Apex.

The overall statistical analysis revealed that the amount of N remaining in the crop resides was affected by the N fertilization rate and the respective plant organ left on the field but not by the cultivar (Supplementary Table S1). Therefore, the amount of N remaining in the crop residues was assessed separately for the N fertilization rates and individual plant organs left on the field across the cvs. Apex and Capitol (Figure 10). The main sources for the remaining N in the crop residues were primarily the pod-walls but also the stems which accounted together for 73% (N0) and 78% (N160) of the crop-residue N. The N remaining in the taproot was the next important crop-residue N source particularly under N limitation (N0). The quantitative contribution of N in intact and shed leaves was low under both N fertilization regimes.
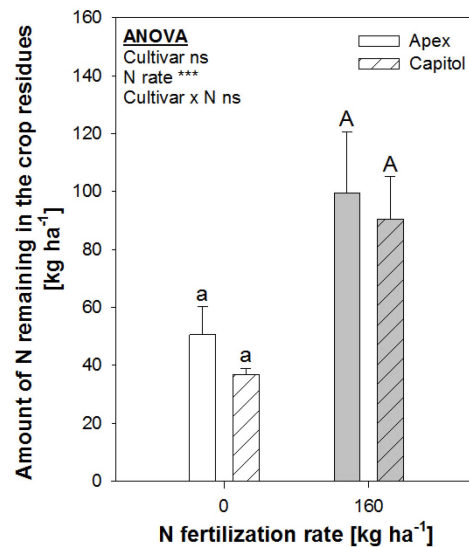

Figure 9. Amount of N remaining in the crop residues (taproot, stem, intact leaves, shed leaves and pod-wall) at maturity of the winter oilseed-rape line-cultivars Apex and Capitol as affected by the N fertilization rate (N0 and N160). Different lower case and upper case letters on the top of the columns indicate differences between the cultivars within the N fertilizer rates at $p < 0.05$. ANOVA: ******* indicates significant differences $p < 0.001$. ns = non-significant. The error bars represent the standard deviations of the means (n = 3–4).
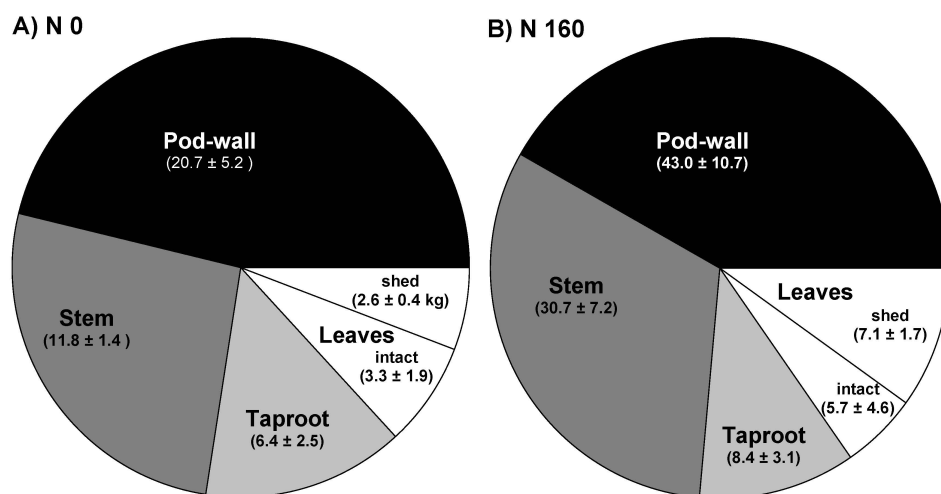

Figure 10. Amount of N (kg N·ha^{-1} ±SD) and distribution among plant parts in the crop residues at maturity (**A**) without N (N0) and (**B**) at optimal N fertilization (N160). The data are shown across the winter oilseed-rape line-cultivars Apex and Capitol (n = 3–4).

3. Discussion

Under favorable environmental conditions winter oilseed-rape line-cultivars usually achieve a grain yield between 4 and 5 t·ha^{-1} [11,23]. In the present field experiment N fertilization significantly increased grain yield formation (Figure 2A). But even under sufficient N supply (N160) a grain yield of 2.7 t·ha^{-1} on average was still low. For grain yield formation the most important yield component in winter oilseed-rape is the number of pods per area, which is a combination of number of plants per area and number of pods per plant [24]. The canopy density in spring was reduced by about 50% to about 30 plants·m^{-2}. This was most likely due to the hard frosts during winter. But winter oilseed rape canopies with 20 to 30 plants·m^{-2} can achieve comparable grain yields to canopies with 70 to 80 plants·m^{-2} [25]. In winter oilseed-rape the high compensation capacity for lower plant density by branching is of major importance for yield stability [26]. But for the exploitation of the yield potential the plant development before winter is decisive [27,28]. The yield potential and winter hardiness of an individual plant depends on biomass formation during autumn. Both are decisively affected by the time of sowing [29,30]. Late sowing in September often results in lower and more variable grain yields [25,28,31,32]. Thus, the late sowing date in September most likely led to an inferior plant growth before winter, which resulted in low grain yields, because of high plant losses during winter in combination with the low yield compensation capacity of the remaining plants.

Without N fertilization (N0) the N deficiency stress was severe with 55% yield reduction across the cultivars (Figure 2A). Under N-limiting conditions (N0) the two cultivars differed in grain yield formation and thus N efficiency according to the definition by Graham [33] and Sattelmacher *et al.* [6] as the ability of a cultivar/genotype to produce a high grain yield under conditions of limited N supply. Without N fertilization (N0) the cv. Apex produced a significantly higher grain yield compared to cv. Capitol. Thus, the reported cultivar differences in N efficiency for the cvs. Apex (N-efficient) and Capitol (N-inefficient) [15,16] could be confirmed. The significantly higher harvest index (HI) of the N-efficient cv. Apex compared to the N-inefficient cv. Capitol revealed that genotypic variation in N efficiency was at least partially based on a higher biomass allocation efficiency to the grains (Figure 3). However, N efficiency of winter oilseed-rape line-cultivars has been primarily attributed to high N uptake efficiency (NUPT), which mainly results from maintained N uptake during reproductive growth [9,10,12]. Under N-limiting conditions (N0) the N-efficient cv. Apex showed a significantly higher NUPT than the N-inefficient cv. Capitol (Figure 4). The significantly higher NUPT of cv. Apex was based on a prolonged post-flowering N uptake until the early phase of grain maturation (DAFF 42). Nitrogen uptake after entering the reproductive phase is regulated by the sink size [34] and maintenance of assimilate allocation to the roots [35]. In winter oilseed-rape a characteristic of *N*-efficient line cultivars with high NUPT during reproductive growth is a functional stay-green phenotype, expressed as delayed senescence of the older leaves and accompanied by maintenance of the photosynthetic capacity [15]. Thus, it is assumed that maintained photosynthetic capacity particularly of lower leaves through delayed leaf senescence will not only result in a better assimilate supply to the pods but also to the root and consequently enhanced N uptake [36,37]. In the present field, experiment N limitation (N0) prematurely induced senescence (Figure 1). However, particularly under N-limiting conditions (N0), the N-efficient cv. Apex showed a delayed senescence-course of the older leaves on the main stem (stay-green) compared to the N-inefficient cv. Capitol. The stay-green phenotype may also explain the higher HI of the N-efficient cv. Apex (Figure 3).

The second pillar of N efficiency is N utilization efficiency (NUE) [6]. Berry *et al.* [9] and Schulte auf'm Erley *et al.* [10] reported that under severe N limitation NUE played a minor role for N efficiency. Under N-limiting conditions (N0) no significant cultivar differences in NUE occurred between the cultivars. However, with increasing N supply NUE becomes equally or even more important for grain yield formation than NUPT [10,11]. But in the present study, most likely owing to the low yield potential, NUE was similar for both N supplies (N0 and N160) for both cultivars (Figure 8A). Physiological traits which may contribute to NUE are a low grain N concentration and an efficient allocation of N to the grains. The grain protein and grain-meal protein concentrations of the N-efficient

cv. Apex were significantly lower than of the N-inefficient cv. Capitol independent of the N supply (N0 and N160) (Supplementary Figure S1). The lower protein concentrations of grains contributing to N efficiency, however, is equivocal from the grain quality and crop N-balance point of view. Although winter oilseed-rape is an oil crop and the oil yield is the most important economic parameter [38], the remaining oilseed meal is a valuable animal feedstuff and protein source [39]. Also, a lower grain N concentration might leave higher N amounts in crop residues contributing to high N balance-surpluses. Therefore, a high N retranslocation for which the N harvest index (NHI) is an overall indicator, is of greater importance as component of NUE. The NHI was 0.41 and 0.55 for limited (N0) and sufficient N supply (N160), respectively (Figure 8B) and thus in the range as reported by Aufhammer *et al.* [3]. But depending on environment and agronomic practices the N allocation to the grains can be highly efficient and reach a NHI of up to 0.83 [23]. The results do not support the view that higher N allocation efficiency contributed to the higher N utilization efficiency of cv. Apex. But since the shoot N uptake at maturity was significantly higher in cv. Apex than in cv. Capitol (Figure 4) a similar NHI led to a significantly greater grain N content (Figure 6A) of the N-efficient cv. Apex.

Particularly, under N-limiting conditions (N0), high pod N contents were related to high grain yields (compare Figures 2A and 5). The final pod N content is primarily based on the duration of N accumulation. Nitrogen uptake during reproductive growth directly contributes to pod N accumulation [18]. The continued N accumulation of N-efficient cv. Apex led to a significantly higher pod N content compared to the N-inefficient cv. Capitol (Figure 5). Accordingly, the pod-N increase closely coincided with the duration of shoot N uptake during reproductive growth (compare Figures 4 and 5). The calculation of the apparent N uptake for pod N accumulation across the two cultivars revealed that the pod N apparently originating from N uptake after full flowering was greater under N limitation (N0, 42%) than at high N fertilization (N160, 28%) (Table 1). Under N-limiting conditions (N0) post-full-flowering N uptake contributing to pod N content was greater, at least in tendency, for the N-efficient cv. Apex compared to the respective N-inefficient counterpart cv. Capitol (Table 1).

In general, the N uptake after full flowering is not sufficient to meet the N demand of the pods. In winter oilseed-rape around 70% of pod N derives from N remobilization primarily from leaves and stems [18,19]. In the present study, 58% and 72% of pod N apparently originated from N remobilization from full flowering until maturity under low (N0) and high N fertilization (N160), respectively (Table 1). At full flowering the stems and the leaves represented the largest N pools under both N supplies (Figure 7B,C). Owing to the high amount of N apparently remobilized from the stem (Figure 7B), the stem was a major source for apparent N remobilization to the pods. But the N remobilization efficiency from the stems with 56% and 63% for limited (N0) and sufficient N supply (N160), respectively, was comparatively low. Ulas *et al.* [23] reported that the apparent N remobilization from the stem during reproductive growth can reach up 88% particularly under N-limiting conditions. Since leaf N losses with shed leaves were rather low (Figure 7D), the leaves were the second major source for N remobilization to the pods (Figure 7C). The apparent N remobilization from the leaves was about 10% higher compared to the stems, with 66% and 73% for limited (N0) and sufficient N supply (N160), respectively, and thus also substantially lower as reported by Ulas *et al.* [23]. Within the N fertilization levels (N0 and N160) no cultivar differences in the N concentrations of stem and shed leaves occurred at maturity (Figure 7B,D). Thus cultivar differences in the amount of N remobilized from stems and leaves were based on differences in N accumulation during vegetative biomass formation rather than N remobilization efficiency. However, using a different set of cultivars Avice and Etienne [40] and Girondé *et al.* [41] concluded that differences in N remobilization efficiency from vegetative to reproductive organs might be important for overall N efficiency of oilseed rape.

The N remobilization efficiencies of the leaves and the stem did not differ much, particularly under N-limiting conditions (compare Figure 7B,D). But the amount of N remaining in the stem was about twice higher under both N supplies (Figure 10) owing to the higher dry matter of the stems. Less than 10% of the N taken up by the shoot remained in the leaves and did not exceed more than

16 kg N· ha^{-1}. Thus and in contrast to the assumption of a weak remobilization of N from fallen leaves [42], shed leaves did not contribute much to the amount of N in crop residues, confirming the results reported by Ulas *et al.* [23] summarizing the results of a three-years field experiments with four cultivars at three N supply levels.

In agreement with the reported small contribution of N remobilization from the taproot to the pods [18,19] the N pool in the taproot was low compared to the shoot and amounted only to 9% and 5% of the shoot N pool at N0 and N160, respectively (Figure 7A). The taproot was not an important intermediate storage organ for N remobilization, since the N pool during reproductive growth remained generally on a constant level even under severe N limitation (N0). Only under sufficient N supply (N160) the taproot N content slightly but significantly decreased in both cultivars.

The generally higher N concentrations remaining in the taproots (Figure 7A), stems (Figure 7B) and shed leaves (Figure 7D) at maturity under high (N160) compared to low N supply (N0) indicate a sink-limitation of grain yield formation. This was in agreement with the above mentioned lower N remobilization efficiency from vegetative plant parts to the grains compared to previous studies [22]. The sink limitation may be explained by the unfavorable climatic conditions during the main growing period in combination with the delayed development before winter, since improved growing conditions in the spring could not compensate an inferior development in the autumn [29,43].

The residual N in stems and particularly pod-walls contributed mainly to the N amounts remaining in the crop residues independent of the N supply (Figure 10). Based on the changes in the N contents of the pod-walls during grain filling (Figure 6B) the N remobilization efficiency could be calculated. Across the two cultivars without N fertilization hardly any N remobilization occurred from the pod-walls during grain filling (2%), whereas at N160 30% of the pod-N was remobilized for transport to the grains. Thus more than two-thirds of pod-wall N was apparently not translocated to the grains. The lower N remobilization efficiency of pod-walls compared to stems and leaves might be due to a limited N requirement and storage capacity for N in the grains, which primarily store oil rather than proteins. However, whether enhancing N remobilization from pod walls to the grains with the objective to reduce the N content in crop residues and thus decreasing the N budget surplus of the winter oilseed-rape crop is possible without compromising oil yield as suggested by Zhao *et al.* [44] and Stahl *et al.* [45] awaits clarification in the future.

4. Experimental Section

4.1. Plant Material and Growing Conditions

For investigating the role of stay-green, N retranslocation and N uptake during the reproductive phase for grain yield formation under N limitation the two line-cultivars Apex and Capitol were selected for the field experiment since previous field experiments classified cv. Apex as N-efficient and cv. Capitol as N-inefficient [15]. The seeds were received from the Norddeutsche Pflanzenzucht Hans-Georg Lembke KG. The field experiment was performed in the year 2011 at the experimental station Poppenburg of the Landwirtschaftskammer Niedersachsen on a loess soil in Nordstemmen, Germany. The cultivars were grown under two N fertilizer rates, without mineral N (N0) and 160 kg N· ha^{-1} (N160) as calcium ammonium nitrate. The soil mineral N content (N$_{min}$) in autumn 2010 before N fertilizer application was N$_{min}$ = 75 kg N· ha^{-1} for a soil depth of 0.9 m. The experiment was laid out in a block design with the N fertilization levels as blocks. Within each block the cultivars were completely randomized in four replicates. The plot size was 12.0 m × 1.5 m (18 m^2). The seeds were sown on 6 September 2010 with a target plant density of 60 plants· m^{-2}. The plant distance between the rows was 25 cm and within a row 12 cm. Due to the hard frosts in winter 2010/2011, the actual plant density in spring was only 30 plants· m^{-2}.

4.2. Plant Harvest and Analysis

The developmental stage of the plants was determined using the BBCH-code (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) [46]. From full flowering (BBCH65; 50% of the flowers on the main raceme open) until maturity (BBCH89) in weekly intervals 0.75 m^2 of each plot were harvested. At the head ends, the plots were shortened by one meter and the border plants (0.5 m) were discarded. The plants were separated into stems, leaves, pods, grains and pod walls. Furthermore the shed leaves were weekly collected and the taproots were dug out. The shoot represented the entire aboveground plant parts: stem, shed leaves, intact leaves and pods. From BBCH79 on (all pods have reached the cultivar-specific size) pod walls and grains were separated. The straw represented the same aboveground plant organs as for the shoot excluding the grains. N concentrations of the dried and ground plant material were determined using an elemental analyzer (Vario EL, Elementar Analysensysteme, Hanau, Germany). At maturity the oil concentration in the grain and the protein concentration in the grains and grain-meal were determined by near infrared reflectance spectroscopy (NIRS) according to the method of Tkachuk [47]. In addition to the destructive harvests, the senescence status of the plant was assessed for three previously marked plants per plot by counting the number of remaining green leaves on the main stem at thirteen time points from full flowering (BBCH65) until 52 DAFF, near maturity (BBCH89). Further agronomic relevant traits and apparent N remobilization and uptake at maturity were calculated based on the following equations:

$$\text{N uptake (NUPT)} = \text{N}_{\text{Straw}} \left[\text{kg·ha}^{-1}\right] + \text{N}_{\text{Grain}} \left[\text{kg·ha}^{-1}\right] \tag{1}$$

$$\text{N utilization (NUE)} = \frac{\text{Grain dry weight [kg·ha}^{-1}]}{\text{N}_{\text{Shoot}} \text{ [kg ha}^{-1}]} \tag{2}$$

$$\text{N harvest index (NHI)} = \frac{\text{N}_{\text{Grain}} \text{ [kg·ha}^{-1}]}{\text{N}_{\text{Shoot}} \text{ [kg·ha}^{-1}]} \tag{3}$$

$$\text{N harvest index (NHI)} = \frac{\text{N}_{\text{Grain}} \text{ [kg·ha}^{-1}]}{\text{N}_{\text{Shoot}} \text{ [kg·ha}^{-1}]} \tag{4}$$

$$\begin{aligned}&\text{Apparent taproot N remobilization}_{\text{BBCH89}} \\ &= \text{Taproot N}_{\text{BBCH89}} \left[\text{kg·ha}^{-1}\right] - \text{Taproot N}_{\text{BBCH65}} \left[\text{kg·ha}^{-1}\right]\end{aligned} \tag{5}$$

$$\begin{aligned}&\text{Apparent stem N remobilization}_{\text{BBCH89}} \\ &= \text{Stem N}_{\text{BBCH89}} \left[\text{kg·ha}^{-1}\right] - \text{Stem N}_{\text{BBCH65}} \left[\text{kg ha}^{-1}\right]\end{aligned} \tag{6}$$

$$\begin{aligned}&\text{Apparent leaf N remobilization}_{\text{BBCH89}} \\ &= \text{Intact leaf N}_{\text{BBCH89}} \left[\text{kg·ha}^{-1}\right] - (\text{Intact leaf N}_{\text{BBCH65}} \left[\text{kg·ha}^{-1}\right] \\ &+\text{Shed leaf N}_{\text{BBCH89}} \left[\text{kg·ha}^{-1}\right])\end{aligned} \tag{7}$$

$$\begin{aligned}&\text{Apparent N remobilization to the pods} \\ &= \text{Apparent taproot N remobilization} \left[\text{kg·ha}^{-1}\right] \\ &+\text{Apparent stem N remobilization} \left[\text{kg·ha}^{-1}\right] \\ &+\text{Apparent leaf N remobilization} \left[\text{kg·ha}^{-1}\right]\end{aligned} \tag{8}$$

$$\begin{aligned}&\text{Apparent N uptake of the pods} \\ &= \text{Pod N}_{\text{BBCH89}} \left[\text{kg·ha}^{-1}\right] - \text{Apparent N remobilization to the pods} \left[\text{kg·ha}^{-1}\right]\end{aligned} \tag{9}$$

4.3. Statistical Analysis

The statistical analysis of the field experiment was performed using the statistic software SAS version 9.2 (SAS Institute, Cary, NC, USA). For the field trials, the analysis of variance (ANOVA) was calculated using the PROC MIXED procedure. For the ANOVA, the Type III sum of squares was applied, if an unequal number of replicates occurred. For the overall analysis cultivar, N rate, DAF and their interactions were set as fixed factors. For the comparison of the cultivars within the N levels, the LSMEANS statement was used. For each comparison, the linear combinations were defined for the cultivar and the cultivar × N rate interaction using the ESTIMATE statement. For all tests of significance, a *p*-value <0.05 was used and the *p*-values were Bonferroni–Holm adjusted. In the tables and figures, for the F-Test +, *, ** and *** indicate significance at the $p < 0.10$, $p < 0.05$, $p < 0.01$ and $p < 0.001$ level, respectively, and ns = non-significant. The same symbols were used to mark the significance for the correlations. For the comparison of means different letters on top of the columns indicate differences between the columns at $p < 0.05$. Curves were fitted using the graphic software SIGMA PLOT version 11 (Systat software, San Jose, CA, USA).

5. Conclusions

In conclusion, for grain yield formation under N-limiting conditions (N0) as well as sufficient N supply (N160) NUPT was more important than NUE in the present experiment. For genotypic variation in N efficiency NUPT, particularly N uptake during flowering and biomass allocation efficiency to the grains (HI) were decisive. Both were related to delayed senescence of the older leaves (stay-green). For pod N accumulation N remobilization from the vegetative plant parts was more important than N uptake after full flowering. The main sources for the high N amounts remaining in the crop residues at maturity were the pod walls (high N concentrations) and the stems (high biomass). Decreasing the crop-inherent high N budget surplus of oilseed rape requires particularly increasing the N remobilization efficiency of pod-wall N to the grains. Addressing this conclusion, multi-year and -location field experiments with an extended range of cultivars including hybrids are desirable.

Author Contributions: Fabian Koeslin-Findeklee conducted the experiment and evaluated and presented the results. Walter Horst initiated the research and provided the necessary research support and guidance. Fabian Köslin-Findeklee and Walter Horst wrote the manuscript.

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