

# Nitrogen dynamics in field grown oilseed rape. Effect of nitrogen fertilization and genotype

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## Abstract

Despite a high nitrate uptake capacity, the nitrogen use efficiency (NUE) of oilseed rape is weak due to a relatively low N remobilization from vegetative (mostly leaves) to growing parts of the plant. Thus, this crop requires high rate of N fertilization and leaves fall with high N content. In order to reduce the rate of N fertilization and to improve the environmental impact of oilseed rape without decreasing yield, it is crucial to better understand the dynamics of N through the plant. This study presents the effect of contrasted N fertilizations and genotypes on the N dynamics of oilseed rape grown under field conditions.

The experiment was conducted in a field-grown winter oilseed rape under three levels of N dose (0, 100 and 200 kg N ha<sup>-1</sup>) and three genotypes (cv. 'Capitol', 'Pollen' and 'Saturnin'). Plants were sampled frequently from stem extension (GS 2,4) to maturity (GS 9,9) by using a <sup>15</sup>N labelling technique in order to distinguish in each tissue N coming from uptake or remobilization.

N fertilization increased biomass and N accumulation whatever the genotype. The partitioning of N taken up was affected by N fertilization and genotype, whereas N uptake decreased during flowering irrespective of N fertilization and genotype. Whatever the N treatment and the genotype, more than 60% of pods N came from N remobilization of vegetative tissues, green leaves being the main source of remobilized N. The timing and the part of N remobilized from the different vegetative organs depended on the N supply and cultivar. The efficiency of N remobilization in leaves increased with the rate of N fertilization and along an axial gradient from the basal to the uppermost leaves.

Better synchronising leaf N remobilization and grain filling could improve the NUE of oilseed rape.

## Introduction

Oilseed rape can be used for a variety of purposes (oil, alternative fuel, animal feed...), this explains its increasing interest throughout the world. Considerable varietal improvements have been achieved in terms of improving seed yield and disease tolerance. However, despite a high nitrate uptake capacity, the Nitrogen Harvest Index (NHI) of oilseed rape is low. Thus, this crop requires high levels of N fertilization in order to maximize yield and inefficient use of applied N leads to risks of N pollution through leaching and denitrification. Improving crop N management and breeding N efficient genotypes could meet the reduction of N fertilization dose without affecting yield.

In oilseed rape, N uptake strongly increases from stem extension to flowering whereas N accumulation during pod filling is low. Thus, the major fraction of pod N derives from the remobilization of N previously accumulated in vegetative organs and in particular, leaves (Dreccer *et al.*, 2000; Rossato *et al.*, 2001; Malagoli *et al.*, 2005). Although leaves mostly fulfil the N demand of pods, the high N content in fallen leaves undergoes unfinished N remobilization (Malagoli *et al.*, 2005). Leaf fall is of considerable interest because the N remaining in prematurely fallen leaves is lost to dry matter production but may increase nitrate leaching following the mineralization of leaf organic N. The return of N to the soil can reach 100 kg N ha<sup>-1</sup>, even if the crop may take up a small proportion of it in spring (Dejoux *et al.*, 2000).

Although numerous studies on oilseed rape have been reported in the literature, the physiological mechanisms, which explain the efficiency of N dynamics through the plant, remain unclear. The present investigation was therefore carried out to study the effects of contrasted N fertilizations and genotype on the dynamics of N uptake, partitioning and remobilization in field-grown winter oilseed rape under three levels of N dose (0, 100 and 200 kg N ha<sup>-1</sup>).

## Materials and methods

The field experiment was conducted in Grignon (Paris Basin, France) during the 2004 growing season. Three cultivars of winter oilseed crop (*Brassica napus* L. cv. Capitol, Pollen and Saturnin) were sown on a loamy soil on August 27 at a density of 71 plants per m<sup>2</sup> and with a row spacing of 17.5 cm. Cultivars were chosen as a function of their differences in height and time of flowering. The Capitol genotype had been studied as a reference in the laboratory from several years; it exhibits half early flowering and a normal height. The Pollen genotype presents with half late flowering and normal height whereas Saturnin is a semi-dwarf hybrid with half early flowering. Three N treatments were defined: N0, N100 and N200, reflecting the application of 0, 100 and 200 kg N ha<sup>-1</sup>, respectively. N fertilizer was applied as NH<sub>4</sub>NO<sub>3</sub>.

The <sup>15</sup>N labelling experiment was performed between stem extension (GS 2.4) and seed maturity (GS 9.9) according to the methodology described by Malagoli *et al.* (2005). One week before each harvest date, six plants from each treatment and at the same developmental stage were selected inside the canopy. 750 ml of labelled N (1mM K<sup>15</sup>NO<sub>3</sub>, <sup>15</sup>N excess = 10%) was carefully applied around each plant, at the soil surface (about 400 cm<sup>2</sup>). Seven days after <sup>15</sup>N labelling, the plants were harvested. The petiole of each senescing leaf was attached to the stem with a nylon thread so that fallen leaves could be

collected later on according to their position on the plant axis.

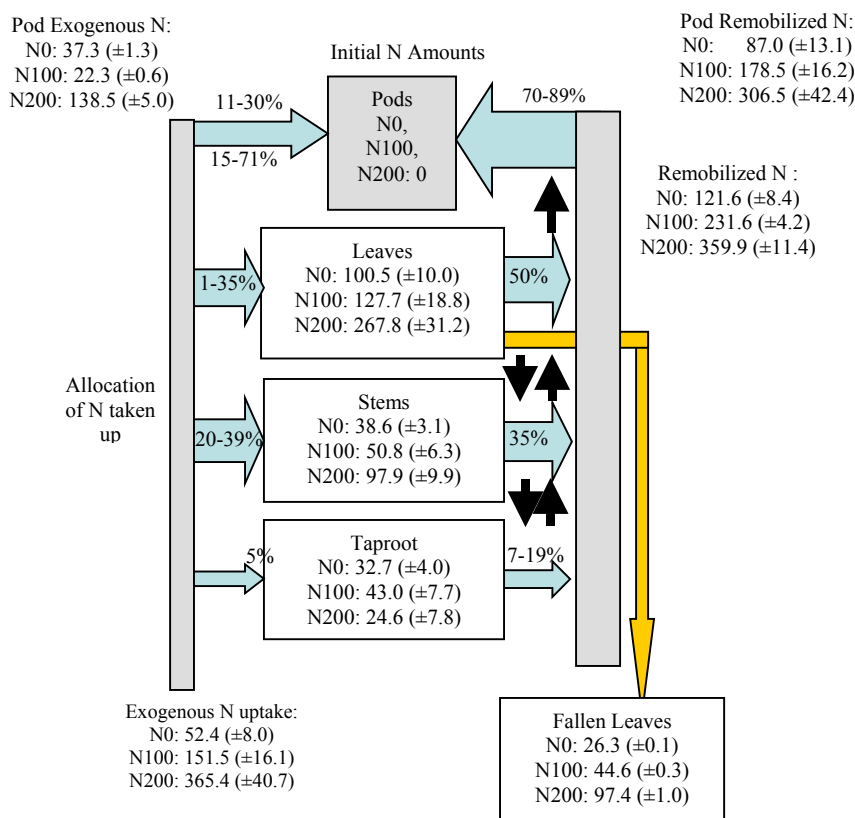
Plants were harvested every third week from the March 4 until the final harvest on July 8. At each sampling, plants were separated into the following 6 fractions: taproot, green and fallen leaves, stems (main stem and axillary racemes), flowers and pods. Because the quantitative study of flowers and lateral roots is difficult under field conditions, roots were omitted from subsequent analyses and flowers were only taken into account for the total plant N amount. Immediately after fractionation, the fresh weight (FW) and total surface area of leaves were measured and dry matter (DM) was determined after drying at 80°C for 48 h.

All experiments were performed on six individual plants. The resulting variations in measurements were expressed as the mean  $\pm$  SD for  $n=6$ . Statistical analyses were performed using Minitab. After checking the normality of the data (Anderson-Darling test, 95%) and the equality of variances (Bartlett test, 95%), treatment and genotype effects were tested by ANOVA (GLM) and the significance of differences was estimated using the Tuckey test (95%). When data did not comply with the parametric test conditions, a non parametric Kruskal Wallis test was applied, followed by the Tukey or Mood median test (95%) to compare means or medians.

## Results

The flow charts (Figures 1 and 2) represent the partitioning of N (taken up and remobilized) through the major organs (pods, leaves, stems and taproot) from stem extension to maturity. Using  $^{15}\text{N}$  labelling, it was possible to follow and quantify the partitioning of N taken up during plant development.

Effect of N fertilization on dynamics of N uptake, partitioning and remobilization



**Figure 1:** Overview of N dynamics in field-grown *Brassica napus* L. 'Capitol' from stem extension (GS 2.4) to maturity (GS 9.9) under three levels of N fertilization: N0 (0 kg N ha<sup>-1</sup>), N100 (100 kg N ha<sup>-1</sup>) and N200 (200 kg N ha<sup>-1</sup>). N amounts are expressed in mg N plant<sup>-1</sup> and each value is given as the mean  $\pm$  standard deviation for  $n=6$ .

The total amount of N taken up during the studied period increased with the rate of N fertilization (52.4, 151.5 and 365.4 mg N plant<sup>-1</sup> respectively in N0, N100 and N200 plants). The partitioning of N taken up through the different organs also varied with the N dose (Figure 1, left arrows). Whereas N taken up by N0 plants was mostly allocated to the pods (71% of the total N taken up), N100 and N200 plants allocated the most N taken up to the stems (39%) and leaves (35%) for N100 and to the pods (38%) and stems (38%) for N200. Whatever the N treatment, the allocation of N taken up to taproot was low (about 5% of the total N uptake).

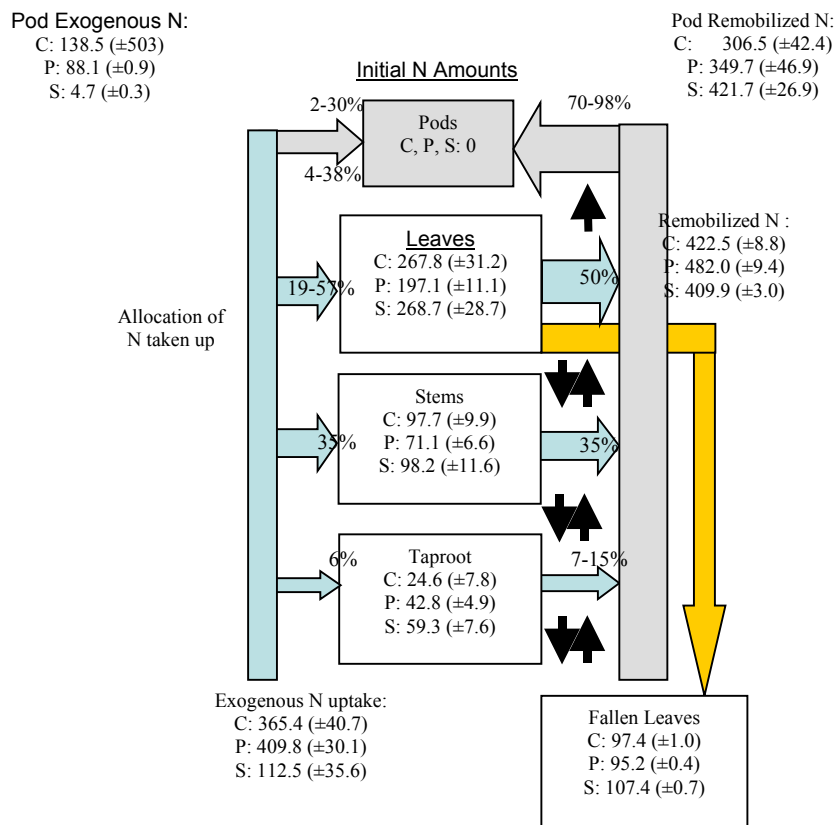
The pool of remobilized N greatly increased with the N supply (150.3, 285.6 and 422.5 mg N plant<sup>-1</sup> in N0, N100 and N200 plants, respectively). Leaves and stems were the main source of remobilized N irrespectively of the N dose, providing around 50% and 35% of the remobilized N pool, respectively (Figure 1, right arrows). The contribution of the taproot declined

with the N dose (from 19% to 7%).

The final N amount in pods (sum of pod exogenous N and pod remobilized N) increased considerably with the N supply (134.3, 200.8 and 445 mg N plant<sup>-1</sup> in N0, N100 and N200 plants, respectively). In N0 and N200 plants, about 70% of pod N came from N remobilization and 30% from N uptake while the relative contribution of N uptake declined to 11% in N100 plants due to a very low post-flowering N uptake.

From stem extension to maturity, the cumulated amount of N lost through leaf fall significantly increased with the N supply from 26.3 mg N plant<sup>-1</sup> in N0 plants to 97.4 mg N plant<sup>-1</sup> in N200 plants, which corresponded approximately to 15 and 41 kg N ha<sup>-1</sup>, respectively.

#### Effect of genotype on dynamics of N uptake, partitioning and remobilization



**Figure 2:** Overview of N dynamics in three field-grown *Brassica napus* L. genotypes ‘Capitol’ (C), ‘Pollen’ (P) and ‘Saturnin’ (S) from stem extension (GS 2.4) to maturity (GS 9.9) under one level of N fertilization: (200 kg N ha<sup>-1</sup>). N amounts are expressed in mg N plant<sup>-1</sup> and each value is given as the mean ± standard deviation for n=6.

Total N uptake greatly varied with genotype. The genotype Saturnin displayed a lower value of exogenous N uptake (112.5 mg N plant<sup>-1</sup>) than the genotypes Capitol and Pollen (365.4 and 409.8 mg N plant<sup>-1</sup>, respectively). Depending on the organ, the partitioning of the exogenous N also varied with genotype. However, N allocation to the stems represented approximately 35% of N uptake irrespective of genotype and N allocation to the taproot was always the lowest of all studied organs (about 6%, Figure 2, left arrows). Differences in N partitioning were observed in pods and leaves with opposite proportions according to the genotype. While the genotype Capitol allocated 38 and 19% of total N taken up to the pods and leaves, respectively, the genotype Saturnin allocated 4 and 57% to the same organs.

The pool of remobilized N was affected by genotype, the genotype Pollen showing a higher pool (482 mg N plant<sup>-1</sup>) than others genotypes. By contrast, the relative contribution of leaves and stems was not affected by genotype. Leaves were the major source of remobilized N, providing more than half of the remobilized N pool. Stems contributed to about 35% of the pool and the contribution of taproot reached 15 and 13%, respectively, in Saturnin and Pollen plants while it represented only 7% in Capitol plants (Figure 2, right arrows).

Although genotypic differences were observed in N uptake and remobilization, the final N amount in pods was not affected by genotype. Whatever the genotype, most N in pods at maturity derived from N remobilization (around 70-98%).

During the studied period, the cumulated amount of N lost in fallen leaves was higher in the genotype Saturnin (107.4 mg N plant<sup>-1</sup>) than in Capitol and Pollen plants (around 96 mg N plant<sup>-1</sup>). These values represented a N lost of 52.5 kg N ha<sup>-1</sup> in Saturnin plants and 41 kg N ha<sup>-1</sup> in Capitol and Pollen plants.

## Discussion and Conclusions

### *N* uptake

N fertilization increased the total N uptake and also affected the partitioning of N taken up. The proportion of N taken up allocated to pods was strongly linked to the pattern of total N uptake. In N100 plants, the extremely low allocation to pods was explained by an absence of post-flowering N uptake. On the contrary, N200 plants displayed a high post-flowering N uptake and thus, allocated a massive part of it to developing pods. Despite significant differences in N uptake during the studied period, which can be explained in part by the sampling date (one week for each genotype), plant N levels at maturity were similar in all genotypes. The absence of post-flowering N uptake in Capitol N100 and Saturnin N200 plants remains unclear. The partitioning of N taken up highly varied with N treatment and genotype and was linked to the pattern of N uptake.

Whatever the genotype and the N treatment, a decline of N uptake was observed at flowering (data not shown), confirming previous studies (Schjoerring *et al.*, 1995; Gabrielle *et al.*, 1998; Rossato *et al.*, 2001; Malagoli *et al.*, 2005). This decline of N uptake could be linked to the drastic decline of the Leaf Area Index (LAI) by more than 50% during flowering (data not shown). The decline of LAI could lead to an imbalance between N source and sink, the N demand becoming weaker than the N supply could reduce N uptake.

### N remobilization

At maturity, 70-89% of pod N derived from the remobilization of N from vegetative tissues. These results confirmed the major effect of N remobilization to N pod filling in oilseed rape (Hocking *et al.*, 1997; Rossato *et al.*, 2001; Chamorro *et al.*, 2002; Malagoli *et al.*, 2005). Green leaves accounted for the largest source of remobilized N (about 50%), irrespective of the N dose and genotype, followed by stems (about 35%) and taproot (less than 20%). However our study did not inform on the precise contribution of each organ to pod N and our too complex field experiment did not allow us to separate pod walls and seeds (except at maturity), but it would have been useful to compare the efficiency of N remobilization of pod walls between genotypes and N treatment.

In spite of their massive contribution to the pool of remobilized N, the amount of N lost through fallen leaves underlined non-optimal leaf N remobilization. The positive effect of N fertilization on cumulated N amount in fallen leaves was explained by larger and more numerous leaves, as well as a higher residual N content (data not shown). Saturnin N200 plants displayed higher cumulated N amounts than others genotypes mainly due to higher residual N content, thus this genotype presented a leaf N remobilization less efficient. Moreover, the major part of leaves fall before the high N demand required for pod development. Thus, a better synchronisation between leaf senescence and pod N filling could improve leaf N remobilization.

As oilseed rape is source-limited for pod growth and yield, a better synchronization of N remobilization from vegetative organs (particularly leaves) during grain filling could improve the N use efficiency of this crop.

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