Dynamics of Accumulation and Partitioning of N in Leaves, Stems and Roots of Lucerne (*Medicago sativa* L.) in a Dense Canopy

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Quantitative relationships were established which defined the partitioning of nitrogen and dry matter between the leaves, stems and roots during the development of field grown lucerne. The coefficients were unaffected by whether or not N fertilizer was applied.

The average % N in the aerial tissue fell sharply with increase in plant mass when aerial DM of the crop exceeded 1 t ha$^{-1}$. This was mainly because the proportion of stems increased and the % N in stems was low. The often-reported decline in % N with decrease in light intensity and increasing depth within the canopy was largely offset by a decline in specific leaf weight, so that the average % N of the leaves declined only slightly as the plants grew.

The average %N of the entire plant (aerial plus root tissues) remained constant at about 2-4 irrespective of plant size. After cutting, regrowth was accompanied by a rapid transfer of up to 40 kgN ha$^{-1}$ from root to shoot, but this loss was made good by N fixation during the main growing period.

Key words: N uptake, N partitioning, leaf/stem ratio, N remobilization.

INTRODUCTION

Previous studies have shown that the decrease of %N of above ground plant material of lucerne during regrowth can be related to the accumulated aerial dry matter (ADM) by an allometric relationship: \[ %N = a(ADM)^b \] (Lemaire et al., 1985) for \( ADM > 1 \) t ha$^{-1}$. As a consequence, the nitrogen accumulated in above ground plant material (N kg ha$^{-1}$) can be related to the aerial plant mass (ADM t ha$^{-1}$) by: \[ N = 10a(ADM)^{1-b} \] The coefficient \( 1-b \) represents the ratio of the relative rate of N accumulation in shoots to that of shoot dry matter. The value of \( 1-b \) is almost constant and close to 0-66 for lucerne (Lemaire et al., 1985) and for other species (Greenwood et al., 1990). Thus the relative rate of nitrogen accumulation in shoots is about two thirds of the relative rate of dry matter accumulation.

The relationship does not take account of the N in roots and therefore does not permit growth rate to be related to nitrogen uptake by the entire plant. The importance of N remobilization from root to shoot after defoliation has been well demonstrated by Ourry, Boucaud and Salette (1988) on ryegrass and by Kim et al. (1991) on lucerne. For a legume, this phenomenon is particularly important because although N$_2$ fixation is severely reduced by shoot removal (Ryle, Powell and Gordon, 1985) leaf regrowth can be supported by remobilization of N reserves.

The decline of N required for shoot growth with increase in crop mass can be attributed to the larger proportion of structural and storage tissues, as these have a lower % N than photosynthetic tissues; the leaf/stem ratio in particular decreases as the plants grow.

Another factor is the redistribution of N from shaded leaves to well illuminated leaves as demonstrated by Hirose and Werger (1987) with *Lysimachia vulgaris* and by Lemaire et al. (1991) with lucerne.

The main purpose of the work described in this paper was to develop quantitative relationships for the dynamics of N and dry matter accumulation at the whole plant level (shoots + roots) of lucerne in a dense canopy, and to elucidate the changes in the pattern of N partitioning between leaves, stems and roots during regrowth after defoliation. A secondary purpose was to investigate the relative importance of each of the two metabolic pathways for N accumulation: N$_2$ assimilation and N$_2$ fixation.

MATERIALS AND METHODS

Two experiments were carried out on two varieties of lucerne, Europe and Vertus.

**Experiment 1**

A crop of lucerne (cv. Europe) was established during the summer of 1987, at Grignon, France. From the spring of 1988, two treatments were applied: N0, mineral nitrogen was withheld, and N+, 140 kg ha$^{-1}$ of mineral nitrogen, in the form of ammonium nitrate, was applied in Feb. and then after each harvest. The experimental plots were set out in three randomized blocks. The crop was destructively harvested by cutting at 6 cm height on 20 May, 12 Jul. and...
for 24 h). A sub-sample was used for determining specific leaf area and leaf area index. In such an experiment it was not possible to measure the total root biomass. During a preceding experiment (Lemaire and Khaity, 1992) it has been established that the tap root biomass in the 0–20 cm layer of soil represented more than 80% of the total root biomass and that the changes in root mass which occur during regrowth after cutting only affected this upper part of the root system. Thus even though the measurement of only this easily accessible part of the root system give lower limits of its total biomass they should give a reliable picture for the study of the relative changes in the quantity of N in the root system during regrowth dynamics.

In the row corresponding to the average sample in aerial biomass, the tap roots to a depth of 20 cm depth were removed from the soil and transferred to the laboratory where they were washed and separated from the 0–6 cm above ground residual plant material. Each fraction was dried at 80 °C for 24 h. Thus samples of harvestable biomass (above 6 cm), leaf and stem fractions, above ground residual biomass (0–6 cm) and tap root biomass (0–20 cm) were obtained for each date of sampling. Total N content of the samples was determined by a Kjeldahl procedure.

Experiment 2

A similar experiment was carried out on two varieties of lucerne (cvs Europe and Vertus) at Lusignan, France, in a split-plot design (variety x nitrogen) with three replications. The crop was established during the spring of 1985. The same procedure of sampling as for expt 1 was used to follow the evolution of harvestable biomass, leaf and stem fractions, above ground residual biomass and tap root biomass (0–20 cm). In this second experiment, the successive harvests were made weekly during two regrowth periods in 1987: P1 refers to regrowth following the cut made on the 29 May; and P2 refers to regrowth following the cut made on the 10 Jul. The N fertilizer treatments were: N0, no mineral N; N+, 90 kg ha⁻¹ applied after each of the two cuts.

The crop was irrigated during the two periods P1 and P2 in order to avoid a reduction in growth because of the development of a soil water deficit.

RESULTS

Only the results of the first experiment are presented in this section. Data from the second experiment are used to test the validity of the model developed in the discussion.

Dry matter growth

Figure 1 shows the increase in harvestable dry matter (Fig. 1A), tap root dry matter (Fig. 1B) and total dry matter (Fig. 1C) during the three regrowth periods of expt 1. N fertilizer had no significant effect on growth. During each period of growth, the increase of harvestable biomass followed a sigmoid curve with a long approximately linear phase. The pattern of increase in tap root biomass was more complex: an accelerated increase during the initial growth...
Accumulation and Partitioning of Nitrogen in Lucerne

Nitrogen accumulation

The accumulation of nitrogen in harvestable biomass, in tap root biomass, and in total biomass over the three periods followed a similar pattern to that for DM accumulation (data not shown). The N+ treatment increased N accumulation in harvestable biomass only during the first growth period and had no effect on N accumulation in the tap root in any of the three periods. The quantity of N accumulated in harvestable biomass (mean of N0 and N+) at the end of each regrowth was approximately 190, 150 and 160 kgN ha\(^{-1}\) over the periods P1, P2 and P3. For the same periods the quantity of N accumulated in total biomass was 270, 230 and 250 kgN ha\(^{-1}\) respectively.

Figure 3 represents the change in the quantity of N in tap roots during the three periods. This decreased at the beginning of the first growth period in spring from 20 to 10 kgN ha\(^{-1}\) and then rose from 10 to 40 kg ha\(^{-1}\) during the last 3 weeks of the period. During the first 3 weeks after each cut the quantity of N in tap roots diminished rapidly by about 30 kgN ha\(^{-1}\) and then during the last 3 weeks of the growth period increased to above the initial value.

The untransformed data of the relationship between nitrogen and dry matter content of the entire plant or components of the plant during all regrowth periods are given in Fig. 4A–D. Allometric relationships between accumulated N in aerial biomass (N\(_A\)) and the weight of aerial biomass (DM\(_A\)); between leaf nitrogen (N\(_L\)) and leaf biomass (DM\(_L\)); and between stem nitrogen (N\(_S\)) and stem biomass (DM\(_S\)) and a linear relationship between total nitrogen (N\(_T\)) and total biomass (DM\(_T\)) fit the data well (Table 1) irrespective of whether fertilizer N was applied or not. The equations of best fit are:

\[
N_A = 75.66 \, \text{DM}^0.658 \\
N_L = 53.79 \, \text{DM}^0.9931 \\
N_S = 31.73 \, \text{DM}^0.9461 \\
N_T = 23.40 + 24.07 \, \text{DM}
\]

The amounts of nitrogen are in kg ha\(^{-1}\) and the weights of dry matter in t ha\(^{-1}\). There was little improvement in the degree of fit by allowing any of the coefficients in any of the equations to vary with N-supply.

Equation (5) indicates that nitrogen accumulated at a constant rate of 24 kg per tonne of total biomass over most of the growing period but the intercept 23.40 indicates that the rate cannot be constant in the early stages of growth (not studied because of insufficient data).

**DISCUSSION**

**Accumulation of N in harvestable biomass**

Equation (2) shows that the relative rate of N accumulation in aerial biomass was approximately equal to...
in the aerial biomass ($N_a$) is related to the weight of aerial biomass by

$$%N_a = 5.16 \times (DM_a)^{-0.29}$$

(6)

The coefficient 5.16 corresponds to the 'initial' %N for $ADM = 1$ t ha$^{-1}$, and the coefficient $-0.29$ represents the shape of the decline in %N. Two hypotheses have been proposed to explain this decline. The first is that as plants become bigger they have a higher proportion of structural tissues which contain little nitrogen. This hypothesis is supported by eqn (1) because it defines a decrease of the leaf/stem ratio with increasing biomass; the stem fraction contains mainly structural components (which have a low %N) and the leaf fraction is mainly photosynthetic components (which have a high %N). By rearranging eqns (3) and (4) we can obtain expressions of the %N of leaf and stem in terms of their biomass as the crops grow.

$$%N_L = 5.38 \times (DM_L)^{-0.079}$$

(7)

$$%N_S = 3.17 \times (DM_S)^{-0.348}$$

(8)

These equations indicate that the 'initial' %N of leaves and stems (for $DM_L$ or $DM_S = 1$ t ha$^{-1}$) are very different, and, in addition, %N of leaves declines only slightly

FIG. 3. Pattern of nitrogen accumulation in tap root biomass of lucerne, for NO (△) and N+ (▲) treatments.

0.70 times the relative growth rate of aerial biomass of the lucerne crop, which is close to the value of two-thirds found by Greenwood et al. (1990) for a large range of C3 and C4 species and is consistent with that predicted by the 'skin and core' hypothesis of Hardwick (1987). From eqn (2) the %N

FIG. 4. Relationship between N accumulation and increase in dry weight for aerial biomass (A), leaf fraction (B), stem fraction (C), and total plant (D) of lucerne during the three growth periods of expt 1 and for NO (△) and N+ (▲) treatments. The fitted equations are given in Table 1.
Figure 5 gives the estimated values plotted against the Leaf Area Index (LAI) of the canopy by:

\[ N_i = 23.56 \text{(LAI)}^{0.78} r^2 = 0.971 \]  (9)

From this relationship we deduce that the quantity of N per unit leaf area \( N_A \) (g m\(^{-2}\)) decreases with increasing LAI according to:

\[ N_A = 2.356 \text{(LAI)}^{0.85} \]  (10)

From the data of the same experiment it was possible to relate the leaf biomass \( (D_{M_L}) \) to the LAI of the crop by a similar relationship:

\[ D_{M_L} = 0.394 \text{(LAI)}^{0.55} r^2 = 0.951 \]  (11)

From this relationship we deduce that the specific leaf weight \( SLW \) (g m\(^{-2}\)) decreases with increasing LAI according to:

\[ SLW = 3.94 \text{(LAI)}^{-0.18} \]  (12)

We therefore conclude that, as expected, the quantity of N per unit leaf area decreased as the canopy developed and a greater proportion of leaves became shaded. This phenomenon is however accompanied by a decrease in SLW which offsets the effect of shading on %N decrease.

Thus the first hypothesis, that as plants age they have a higher proportion of structural tissues with little N, seems to play the major role in the decrease of %N of harvestable biomass of lucerne. The importance of the remobilization of nitrogen from shaded leaves at the base of the canopy to well illuminated leaves at the top which was postulated by Charles-Edwards et al. (1987) as being the cause of the decrease in plant %N appears to be largely counterbalanced by C respiratory losses which lead to a decrease in SLW of shaded leaves. For instance, Lemaire et al. (1991) found a decrease in N content per leaf area from 2.6 to 0.5 g N m\(^{-2}\) between the top and the base of a lucerne canopy, which was accompanied by a decrease in the specific leaf weight from 3.9 to 2.5. The change in leaf/stem ratio described by eqns (1) and (2) plays the major role in the decrease of plant %N.

By combining eqns (1), (7) and (8) it is possible to express \( N_i \) in terms of LAI and the weight SLW (g m\(^{-2}\)) decreases with increasing LAI according to:

\[ N_i = 53.8 \{(1.43 D_M^{0.43} / (1 + 1.43 D_M^{0.5}))^{0.91} \} \]  (13)

\[ N_i = 31.7 \{(D_M / (1 + 1.43 D_M^{0.5}))^{0.961} \} \]  (14)

Thus, the quantity of N in the harvestable biomass of lucerne \( (N_i = N_A + N_N) \) can be directly related to the quantity of harvestable biomass using the sum of eqns (13) and (14). Using this model we have calculated the predicted values of \( N_i \) from the values of \( D_M \) obtained during expt 2. Figure 5 gives the estimated values plotted against the measured values. Neither the intercept nor the gradient of the line of best fit differed significantly from 0 and 1, respectively, the values for perfect agreement. There was no improvement in the variance removed by fitting separate regressions for each level of N fertilizer, therefore the partitioning of N between stems and leaves is largely independent of the nature of the nitrogen source: \( N_i \) fixation or \( NO_3 \) assimilation.

\( N \) in tap roots

The quantity of N in the tap root and in residual biomass after cutting can be deduced directly from eqns (2) and (5) because:

\[ N_i = N_A - N_N \]  (15)

Thus

\[ N_i = (24.07 \times D_M + 23.4) - 51.6 \times (DM_i)^{0.70} \]  (16)

Immediately after the cut, \( D_M = 0 \) and \( D_M = DM_i \); the initial quantity of N in tap roots and residual biomass \( (N_{i0}) \) can be estimated by:

\[ N_{i0} = 24.07 \times D_M + 23.4 \]  (17)

Thus the dynamics of change of N in the tap root and in residual biomass \( (N_i - N_{i0}) \) can be related to the dynamics of change in total biomass \( (D_M - D_M_i) \) and the dynamics of aerial biomass accumulation \( (DM_i) \):

\[ N_i - N_{i0} = 24.07(D_M - D_M_i) - 51.6(DM_i)^{0.70} \]  (18)

The first term of this equation represents the quantity of N newly accumulated in total biomass from the last cut \( (N_i - N_{i0}) \); the second term represents the quantity of N accumulated in aerial biomass \( (N_i) \). From the data of expt 2 we calculated \( N_i - N_{i0} \), \( N_i \) and \( N_i - N_{i0} \). The comparison between observed and estimated values is given in Table 2. The observed values of \( N_i - N_{i0} \) are not well correlated with the estimated values. The gradient of the best fit between observed and estimated values of \( N_i \) is significantly less than 1, and the intercept is not significantly different from 0, indicating a tendency of the model to
TABLE 2. Estimation of the regression parameters between measured values (expt 2) and estimated values [eqn (20)] of \( N_r - N_s \), \( N_r - N_s' \), and \( N_s' \)

<table>
<thead>
<tr>
<th>Regression coefficient</th>
<th>Intercept</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N_r - N_s )</td>
<td>0.449 (±0.109)</td>
<td>2.66 (±4.00)</td>
</tr>
<tr>
<td>( N_r - N_s' )</td>
<td>1.008 (±0.037)</td>
<td>22.12 (±2.44)</td>
</tr>
<tr>
<td>( N_s' )</td>
<td>0.956 (±0.033)</td>
<td>2.93 (±5.17)</td>
</tr>
</tbody>
</table>

Fig. 6. Relationship between change in total N (\( N_r - N_s \)) and change in total biomass (\( DM_r - DM_s \)) of lucerne after cutting during expt 2: \( N_0 (\triangle) \); \( N+ (\triangle) \).

overestimate N by about 4%. The positive intercept of the regression line between observed and estimated \( N_r - N_s \) indicates that the model underestimates the accumulation of N in total biomass. The linear regression between \( N_r - N_s \) and \( DM_r - DM_s \) represented in Fig. 6 shows a positive intercept of 25 kgN ha\(^{-1}\) indicating a higher rate of N accumulation per unit of total biomass increment at the beginning of the regrowth:

\[
N_r - N_s = 25.12 + 23.75(DM_r - DM_s) \quad r^2 = 0.965 \quad (19)
\]

\( DM_r - DM_s \) represents the net increase in total biomass and can be considered as the difference between the new biomass elaboration and the respiration losses of the biomass remaining after the cut (\( DM_s \)). The rate of increase in \( N_r - N_s \) is proportional to the rate of accumulation of the new plant material and the maintenance respiration associated with \( DM_r \) progressively declines asymptotically during regrowth. In these conditions the rate of accumulation of N in total biomass rapidly reaches a constant value of approximately 24 gN kgDM\(^{-1}\) as expected by the model [eqn (5)].

Taking into account eqn (19), eqn (18) becomes:

\[
N_r - N_s = 25.12 + 23.75(DM_r - DM_s) - 51.6(DM_s)^{0.709} \quad (20)
\]

In order to express \( N_r - N_s \) as a unique variable representative of the regrowth dynamics, we have established a relationship between \( DM_r - DM_s \) and \( DM_s \):

\[
DM_r - DM_s = -0.65 + 1.121DM_s \quad r^2 = 0.966 \quad (21)
\]

The negative value of intercept, significantly different from 0, can be interpreted as the consequence of both the maintenance respiration of the biomass remaining after the cut (\( DM_s \)) and the contribution of tap root reserve for shoot regrowth.

Using eqns (20) and (21), it is possible to express directly the change in root biomass (\( N_r - N_s \)) as a function of the dynamics of aerial biomass accumulation (\( DM_s \)) during regrowth:

\[
N_r - N_s = 9.68 + 26.62DM_s - 51.6(DM_s)^{0.709} \quad (22)
\]

Figure 7 represents the time course of N accumulation in both total and aerial biomass during regrowth. The model gives a good estimation of both \( N_r \) and \( N_r - N_s \) with a residual standard error of about 15 kgN ha\(^{-1}\), but the difference between these two variables leads to a precision in
the estimation of \( N_r - N_p \) of the same order of magnitude as that of the amplitude of variation in the observed values. Nevertheless the model simulates the general pattern of change in the quantity of N in tap roots during regrowth. After the cut the quantity of N in roots decreased rapidly until the aerial biomass reached about 2 t ha\(^{-1}\). At this time the lucerne canopy had recovered to an optimum LAI of 3 and we can estimate that the apparent contribution of endogenous N (\( N_r - N_p \)) to shoot regrowth was about 30 kgN ha\(^{-1}\), that corresponds to one-third of the quantity of N necessary for the elaboration of the aerial biomass. During the second part of the regrowth period the N reserves are restored. Using \(^{15}\)N in controlled conditions Kim et al. (1991) obtained a similar pattern of remobilisation of tap root N, but with a wider amplitude: after 10 d of regrowth nearly all the N in leaves and stems was derived from endogenous reserves. These results confirm the importance of the contribution of tap root N reserves to the dynamics of the LAI recovery after cutting lucerne.

In conclusion, these results confirm that as plants become bigger they have a higher proportion of structural tissues (stem) which contain little nitrogen. In consequence the amount of N per kg of shoot mass decreases with increase in plant biomass. Nevertheless during regrowth the weight of N per unit weight of total plant mass, i.e. aerial parts plus roots, remains constant throughout at 0.024 feqn (5). The decrease in the N concentration in the aerial parts is compensated for by an increase in the concentration of N in the tap roots. After cutting, these reserves move to the foliage where they permit LAI recovery, photosynthesis and regrowth.

Such a model could be useful for optimizing the cutting regime of lucerne crop. The rapidity of regrowth of the crop after cutting is largely determined by the N reserves in the tap root, so the model could be used to determine when cutting should take place in order to achieve a maximum recovery of tap root-N by the above-ground parts of plant.

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**LITERATURE CITED**


