P effects on N uptake and remobilization during regrowth of Italian ryegrass (*Lolium multiflorum*)

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Abstract

The influence of P deficiency on the utilization of two sources of N, mineral N (exogenous N) and reserved N (endogenous N), for regrowth of Italian ryegrass (*Lolium multiflorum*) was studied. P-sufficient (+P) or P-free (−P) nutrition solution was applied from 7 days before defoliation to 24 days of regrowth and the N flows derived from two different N sources within the plant were quantified by \(^1\)\(^5\)N pulse-chase labeling. Shoot regrowth significantly reduced by 12 days of regrowth, while root growth was more in −P plants. Inorganic P (P\(_i\)) concentration was highly reduced by P deprivation more in the stubble and regrowing shoots and less in the roots. At defoliation, P deprivation had induced a higher accumulation for all N compounds in the stubble and for amino acids in the roots. The previously incorporated \(^1\)\(^5\)N in stubble and roots as nitrate and amino acids was much decreased in −P plants especially for the first 6 days of regrowth. Total N content in the regrowing leaves was not significantly different between +P and −P plants, but percentage contribution of remobilized N for total leaf N formation was significantly higher in −P plants (78%) than in +P plants (69%) at 6 days of regrowth. From day 12, the utilization of both endogenous and exogenous N was apparently inhibited in −P plants.

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1. Introduction

Phosphorus is an essential element for higher plants and required in substantial concentration in plant tissues, particularly during vegetative growth. Suboptimal phosphorus supply diminishes
photosynthetic CO₂-fixation rates (Terry and Ulrich, 1973), the expansion of leaf surface (Radin and Eidenbock, 1984), and the number of emerging leaves (Lynch et al., 1991). It may also lead to decreased cytosolic orthophosphate (Pᵢ) levels (Treeby et al., 1987). Orthophosphate is thought to regulate the activities of several enzymes involved in starch and sucrose metabolism (Freddeen et al., 1989). Much of the evidence in support of Pᵢ as a key regulator of carbon partitioning has been obtained (Freddeen et al., 1989; Rychter and Randall, 1994). In terms of dry matter yield, the root is less affected than the shoot so that P-deficient plants are typically low in shoot-to-root dry weight ratio (Freddeen et al., 1989; Heuwinkel et al., 1992). The lower ratio appears to relate to preferential partitioning of carbohydrate towards roots (Khamis et al., 1990). Other features of P-deficient plants include: (i) a marked depression of NO₃⁻ uptake (Heuwinkel et al., 1992; Pilbeam et al., 1993) possibly due to decreased availability of ATP and a limitation in the synthesis of membrane transport system for NO₃⁻ (Rufty et al., 1993); (ii) decrease in translocation of NO₃⁻ from the roots to the aerial part (Pilbeam et al., 1993; Jeschke et al., 1997) related to a fall in water pressure via the root and xylem (Rufty et al., 1993); and (iii) increase in amino acid levels (Rufty et al., 1993; Jeschke et al., 1996, 1997). These factors lead to a limitation of mineral N utilization.

Regrowth of grass species was dependent on two sources of N: one is N reserves previously incorporated in stubble and roots (endogenous N) and the other is mineral N taken up from soil (exogenous N). Several studies (Ourry et al., 1988; Thornton et al., 1993; Louahlia et al., 1999) have shown that different grass species, including Lolium perenne, Festuca rubra, Agrostis castellana, and Poa trivialis, are able to remobilize endogenous N to sustain early regrowth. Isotope labeling works (Ourry et al., 1988; Kim et al., 1991, 1993) have provided the evidences that N allocated to shoot regrowth is predominately mobilized from roots and stubble, since N uptake from soil is restricted for the early regrowth period. Thus, adequate P nutrition could be a crucial factor for shoot regrowth. This is based on a view that P nutritional status actively affects the utilization of two N sources available for shoot regrowth.

The influence of P nutrition on carbohydrate and nitrogen metabolism has been examined in various crop species mainly in intact plants (Freddeen et al., 1989; Pilbeam et al., 1993; Jeschke et al., 1996, 1997), but much less in regrowing plants after defoliation. Furthermore, less is known about how does P nutrition affect the N utilization of N reserves in the remaining organs (stubble and roots) and/or mineral N taken up from nutrient medium for shoot regrowth.

The objective of this work is to test the hypothesis that (1) the P nutrition before defoliation has a significant influence on the accumulation of N reserves in stubbles and roots and (2) contribution of N reserves and mineral N for the production of new foliage is differently responded to the pool size of N reserves and P nutrition during the period of regrowth. A pulse-chase ¹⁵N labeling technique, permitting quantitative analysis of the partitioning of two N sources (N derived from reserves or from mineral uptake), was applied to the Italian ryegrass plants grown with P-sufficient or P-deprived medium for 7 days before defoliation and for 24 days of regrowth.

2. Materials and methods

2.1. Plant culture and sampling

Italian ryegrass (Lolium multiflorum L.) seeds were germinated in a sand bench. Five seedlings at the three-leaf stage were transferred to 3-l plastic pots filled with complete nutrient solution containing, in mM, 4.0 NO₃⁻, 3.0 PO₄³⁻, 3.0 K⁺, 1.5 Ca²⁺, 1.5 Mg²⁺, 3.0 SO₄²⁻, 3.0 Cl⁻, and micronutrients (Fe, B, Mn, Zn, Cu, and Mo). CaCO₃ was then given at a final concentration of 2.0 mM in order to maintain the solution pH at 6.5±0.4. The culture medium was continuously aerated and changed every 6 days. The thermoperiod was 25 °C (day) and 20 °C (night). Light was provided by high-pressure sodium lamps supplying approximately 250 μmol m⁻² s⁻¹ at the height of canopy for 16 h per day. All plants were grown through
two regrowth cycles before treatments were imposed, to allow the plants to develop a satisfactory tillering and root size. When the plants developed at full-vegetative stage (16-week-old), 20 pots (four sampling dates × five replicates) were fed with 3 mM KH$_2$PO$_4$ (P-sufficient, +P), while other 20 pots received a P-free nutrient solution (P-deprived, −P) from 7 days before defoliation. The P treatments continued for 24 days of regrowth. Sulfate and chloride were chosen as substituting P treatments.

4.0 mM Na$_{15}$NO$_3$ at 4000 g for 10 min and passed through an H$^+$ column (Dowex 50W, Sigma). The pH of the solution that passed through the H$^+$ column was adjusted to 7.0 by the addition of 1 N NaOH solution. This solution was concentrated to a final volume of 0.5 ml and corresponded to the nitrate fraction. Amino acids were eluted from the H$^+$ column with 25 ml of 0.5 N HCl and concentrated to 1.0 ml. The dried ethanol-insoluble fraction of the sample was treated with protease from Streptomyces griseus (type IV, Sigma) at 30 °C for 24 h in a 100 mM Na phosphate buffer (pH 7.5) as previously described by Avice et al. (1996). The supernatant, containing amino acids and peptides from proteins, was concentrated to 1.0 ml. This fraction corresponded to proteins. Concentrating was done by drying each collected solution by rotary vacuum evaporation and redissolving the residues in distilled water to obtain the final volume of each fraction as described above. One to five milligrams of freeze-dried powder samples were weighed (precision: 10 μg) into tin capsules for total N determination. For the liquid samples fractionated as described above, an appropriate sample volume, usually 0.1 ml, containing more than the minimum quantities (25 μg N), was dropped into a tin capsule which had been cooled with liquid nitrogen. The tin capsules were then dried in a freeze dryer. The N content and the $^{15}$N at.% of all fractions were determined using N single mode analysis on an ANCA-SL mass spectrometer (Europa Scientific, Crewe, UK).

2.2. P chemical fractionation and P analysis

Total phosphorus determination was made on perchloric:nitric (1:1, v/v) acid digests of freeze-dried tissues using a sensitive method for P determination (Lanzetta et al., 1979). Inorganic P (Pi) was estimated by homogenizing in 2% acetic acid and extracting for 30 min before centrifuging at 4000 × g for 10 min (Freden et al., 1989). Organic P was calculated by subtracting inorganic P from total P.

2.3. N chemical fractionation and $^{15}$N analysis

About 200 mg of finely ground freeze-dried sample was mixed in 25 ml of 80% ethanol (v/v) and heated on a hot plate for 5 min. The ethanol-soluble fraction, which contained NO$_3^-$ and amino acids, was centrifuged at 4000 × g for 10 min and passed through an H$^+$ column (Dowex 50W, Sigma). The pH of the solution that passed through the H$^+$ column was adjusted to 7.0 by the addition of 1 N NaOH solution. This solution was concentrated to a final volume of 0.5 ml and corresponded to the nitrate fraction. Amino acids were eluted from the H$^+$ column with 25 ml of 0.5 N HCl and concentrated to 1.0 ml. The dried ethanol-insoluble fraction of the sample was treated with protease from Streptomyces griseus (type IV, Sigma) at 30 °C for 24 h in a 100 mM Na phosphate buffer (pH 7.5) as previously described by Avice et al. (1996). The supernatant, containing amino acids and peptides from proteins, was concentrated to 1.0 ml. This fraction corresponded to proteins. Concentrating was done by drying each collected solution by rotary vacuum evaporation and redissolving the residues in distilled water to obtain the final volume of each fraction as described above. One to five milligrams of freeze-dried powder samples were weighed (precision: 10 μg) into tin capsules for total N determination. For the liquid samples fractionated as described above, an appropriate sample volume, usually 0.1 ml, containing more than the minimum quantities (25 μg N), was dropped into a tin capsule which had been cooled with liquid nitrogen. The tin capsules were then dried in a freeze dryer. The N content and the $^{15}$N at.% of all fractions were determined using N single mode analysis on an ANCA-SL mass spectrometer (Europa Scientific, Crewe, UK).

2.4. Calculation of N flows

The calculations performed to estimate net N flows from $^{15}$N data are based on various assumptions, many of which are supported by other works and have been summarized by Kim et al. (1991) and Avice et al. (1996). Briefly, the apparent change (dN/dt) in N content in a plant organ during regrowth is the difference between N inflow and N outflow from this organ:

$$\frac{dN}{dt} = N_{\text{inflow}} - N_{\text{outflow}} = N_{t} + dN - N_{t} \quad (1)$$

where $N_{t}$ and $N_{t+dN}$ are N contents at time $t$ and $t + dt$, respectively.
Because plants were fed with inorganic $^{14}\text{N}$ during regrowth period, the increase in $^{14}\text{N}$ content was derived from mineral N uptake. Thus, N inflow derived from N uptake during $dt$ can be calculated from $^{15}\text{N}$ dilution as:

$$\text{N inflow} = \frac{N_{t+dt} \left( 1 - \frac{E_{t+dt}}{E_t} \right)}{C_{30}}$$

where $E_t$ and $E_{t+dt}$ are at.% $^{15}\text{N}$ excess in the organ N measured at time $t$ and $t+dt$, respectively. N outflow from a plant organ, corresponding to endogenous N remobilization during $dt$, can therefore be calculated from Eq. (1):

$$\text{N outflow} = N_t - N_{t+dt} + \text{N inflow}$$

Substitution from Eq. (2) gives:

$$\text{N outflow} = \frac{(N_t \times E_t) - (N_{t+dt} \times E_{t+dt})}{E_t}$$

2.5. Statistical analysis

One-way ANOVA was applied at every time course of regrowth measured in order to clarify the P effects. Where the $F$-test was significant ($P < 0.05$) the differences were determined using Tukey’s Studentized Range test (Steel and Torrie, 1980).

3. Results

3.1. Dry matter

Dry matter accumulation in regrowing leaves did not differ by P treatment until 6 days of regrowth, thereafter it was greatly depressed in the $-\text{P}$ plants (Table 1). It was estimated that P deprivation depressed new foliage by 22.5% at day 24. The changes in dry weights of the remaining organs (stubble and roots) were small for 12 days of regrowth on the $+\text{P}$ medium. However, a large decrease in the dry weight of stubble occurred in the $-\text{P}$ plants for the first 6 days of regrowth. Compared with $+\text{P}$ plants, the dry weight of stubble in $-\text{P}$ plants was significantly smaller for all periods of regrowth, while that of roots was not significantly changed until day 12 and slightly higher at day 24. The shoot (leaves + stubble):root ratio increased progressively in both P treatments. The ratio was remarkably higher in the $+\text{P}$ plants from 12 days of regrowth, mainly due to new tiller formation.

3.2. Tissue P

Phosphorus concentrations in leaves, stubble, and roots changed with P treatments (Fig. 1). Total leaf P and inorganic P concentrations slightly increased in the $+\text{P}$ plants, while it gradually decreased in $-\text{P}$ plants as regrowth proceeded. Total P and inorganic P concentrations in stubble were the highest among three plant parts in both $+\text{P}$ and $-\text{P}$ plants at each sampling date. In $+\text{P}$ plants, inorganic P accounted for about 73–86% of total stubble P. The decreases of total P and inorganic P in the stubble of $-\text{P}$ plants were the largest among three plant parts and reached 33.5 and 17.9% of the $+\text{P}$ plants, respectively, at day 24. P concentrations in both $+\text{P}$ and $-\text{P}$ plants progressively decreased for 24 days of regrowth. It appeared that in roots of $-\text{P}$ plants, organic P was the largest P compound. The difference in total P concentration between $+\text{P}$ and $-\text{P}$ plants was the smallest in the roots among three plant parts.

3.3. Distribution of $^{15}\text{N}$ to different biochemical pools during regrowth period

The changes in $^{15}\text{N}$ excess content of nitrogenous fractions in the remaining organs (stubble and roots) during 24 days of regrowth after defoliation are shown in Fig. 2. The amount of $^{15}\text{N}$ nitrate in stubble at day 0 was 760 and 1150 µg per plant on the $+\text{P}$ and $-\text{P}$ medium, respectively, and largely decreased during regrowth. The decreasing rate of $^{15}\text{N}$ nitrate in stubble of the $-\text{P}$ plants was higher for the first 6 days and then less varied compared with $+\text{P}$ plants (Fig. 2A). The $^{15}\text{N}$ nitrate in roots was not significantly affected by 7 days of P treatment before defoliation (Fig. 2B). A distinctly higher decrease of $^{15}\text{N}$ amino acids in stubble (Fig. 2C) and roots (Fig. 2D) of the P-deprived plants.
was observed for the first 6 days of regrowth. During this period, 62.5 and 63% of the initial level (day 0) decreased in stubble and roots for the +P plants, while remained at same level in stubble and decreased by 57.5% in roots for the -P plants. The initial amounts of 15N proteins were 859 and 1112 mg per plant in /C27/P and /C28/P stubble, respectively, corresponding about 2/3 times larger than those of roots. It decreased with a nearly same rate in both P treatments until day 12, and no prolonged decrease was observed only in stubble of -P plants (Fig. 2E). The initial amounts of 15N proteins in roots were not significantly different between /C27/P and /C28/P plants. A significantly lower decrease in /C28/P plants, for the first 6 days, was exceptionally observed (Fig. 2F), showing no stimulated remobilization of 15N proteins by P deprivation.

The changes in 15N excess content in nitrogenous fractions in regrowing leaves during 24 days of regrowth are shown in Fig. 3. The amount of 15N nitrate continued to increase on the +P medium, while it was leveled-off by P deprivation from day 6 (Fig. 3A). At day 24, the amount of 15N nitrate in -P plants was only 45% of +P plants. The amount of 15N amino acids showed a similar pattern with 15N nitrate response to P treatment (a continuous increase in the +P leaves and no more increase from day 12 in the -P leaves) (Fig. 3B). The pool of 15N proteins was the largest of N compounds examined, suggesting that the 15N remobilized from the remaining organs mainly incorporated to proteins in both +P and -P plants. It increased linearly to 2.1 mg per plant in +P plants, but staggered from day 12 in -P plants (Fig. 3C). After 24 days of regrowth, total

Table 1
Changes in dry weight (mg per plant) of regrowing leaves and remaining organ (stubble and roots) of +P or -P plants during 24 days of regrowth

<table>
<thead>
<tr>
<th>Days of regrowth</th>
<th>Leaves (L)</th>
<th>Stubble (S)</th>
<th>Roots (R)</th>
<th>(L+S)/R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+P</td>
<td>-P</td>
<td>+P</td>
<td>-P</td>
</tr>
<tr>
<td>0</td>
<td>–</td>
<td>–</td>
<td>1200 bA</td>
<td>1170 abA</td>
</tr>
<tr>
<td>6</td>
<td>344 cA</td>
<td>320 cA</td>
<td>1050 cA</td>
<td>888 cB</td>
</tr>
<tr>
<td>12</td>
<td>1010 bA</td>
<td>735 bB</td>
<td>1190 bA</td>
<td>956 cB</td>
</tr>
<tr>
<td>24</td>
<td>2430 aA</td>
<td>1880 aB</td>
<td>1480 aA</td>
<td>1270 aB</td>
</tr>
</tbody>
</table>

The values are means of five replicates. Means followed by the same capital letter in the rows or small letter in the columns, for each plant organ, are not significantly different at P = 0.05.

Fig. 1. Changes in phosphorus concentration in regrowing leaves, stubble, and roots of +P or -P plants during 24 days of regrowth. Mean values labeled with the same capital letter for inorganic P (P_i) or small letter for organic P are not significantly different (P ≤ 0.05, n = 5).
$^{15}$N content in regrowing leaves on the $-P$ medium was 37.4% lower than that on the $+P$ medium.

3.4. Origin of regrowing leaves N

Considering the N and $^{15}$N data analyzed, and using Eqs. (2) and (4) described previously in Section 2, the origin of nitrogen in regrowing leaves was separated into that derived from N reserves in remaining organs and that from mineral N uptake, which was subsequently translocated to the regrowing leaves (Fig. 4). For the first 6 days, leaf N was not significantly affected by P treatments and it was mainly (78.0% for the $-P$ plants and 69.5% for the $+P$ plants) derived from mobilization of N reserves. Absolute amount of remobilized N from remaining organs was 12.6% higher in $-P$ plants during this period. The N content at day 12 was decreased by P deprivation. The amount of N remobilized from reserves during this regrowth period was nearly the same in both treatments. Decline of N content in the $-P$ plants was mainly due to a significantly lower translocation of newly absorbed N from the medium. At day 24, a large proportion of leaf N (69.6% in the $+P$ medium and 73.1% in the $-P$ medium) was attributed to translocation of mineral N. P deprivation significantly decreased the utilization of both N sources for regrowth, showing 28.5 and

![Image](image_url)

Fig. 2. Changes in amounts of $^{15}$N excess of nitrate (A, B), amino acids (C, D), and protein (E, F) fractions of stubble (A, C, E) and roots (B, D, F) of $+P$ (○) or $-P$ (●) plants during 24 days of regrowth. Each value is given as the mean ± S.E. for $n = 5$.

![Image](image_url)

Fig. 3. Changes in amount of $^{15}$N excess of nitrate (A), amino acids (B), and protein (C) fractions in regrowing leaves of $+P$ (○) or $-P$ (●) plants during 24 days of regrowth. Each value is given as the mean ± S.E. for $n = 5$. 
15.3% of decrease in N remobilized from reserves and N translocated from the medium, respectively, in the /C28/P plants.

3.5. Partitioning of N reserves and mineral N

The partitioning of remobilized endogenous N and translocated exogenous N within the regrowing plants on the −P medium over 24 days was compared with the plants regrown on the +P medium (Fig. 5). N content in all three organs was significantly lower in the −P plants. Total mineral N uptake from the P-deprived medium decreased by 13.7% compared with +P plants. The percentage of mineral N distribution into each organ was unaffected by P treatments. However, the absolute translocation of mineral N to leaves and stubble significantly decreased on the −P medium. The regrowing leaves were the only strong sink for endogenous N reserves. About 66.2 and 63.8% of N remobilized to regrowing leaves were derived from stubble in the +P and −P plants, respectively, indicating that stubble was a main source for N reserves. For 24 days of shoot regrowth, the contributions of N reserves in stubble and roots on the −P medium were 31.0 and 23.4% lower than those on the +P medium.

4. Discussion

The depression of shoot regrowth was more noticeable with longer P deprivation (Table 1). Non-significant difference in shoot regrowth between +P and −P plants for the first 6 days may be associated with a low uptake of external nutrients and a large mobilization of the reserves as previously found in other forage species (Ourry et al., 1988; Kim et al., 1991, 1993; Louahlia et al., 1999). P-deprived (−P) plants started to display the symptoms of deficiency after 12 days of regrowth (Table 1), when external nutrients taken up from the medium become an effective source for growth of new leaves (Fig. 4). Regrowth of −P plants was characterized by a stimulated increase in root dry weight and a depressed increase in

\[
\text{N uptake} = 99.4 \pm 6.6
\]

\[
\text{Regrowing leaves} = 91.5 \pm 3.4
\]

\[
\text{Stubbles} = 18.4 \pm 2.2
\]

\[
\text{Roots} = 9.4 \pm 0.3
\]

\[
\text{N uptake} = 85.8 \pm 6.6
\]

\[
\text{Regrowing leaves} = 73.8 \pm 6.1
\]

\[
\text{Stubbles} = 23.8 \pm 1.3
\]

\[
\text{Roots} = 6.1 \pm 0.1
\]

\[
\text{N uptake} = 19.9 \pm 1.7
\]

\[
\text{Regrowing leaves} = 12.7 \pm 2.4
\]

\[
\text{Stubbles} = 7.2 \pm 0.7
\]

\[
\text{Roots} = 7.2 \pm 0.7
\]

Fig. 4. Total nitrogen increments in regrowing leaves of +P or −P plants over 24 days of regrowth. Black and white bars indicate the N derived from remobilization of N reserves and from mineral N uptake, respectively. Mean values labeled with the same capital letter for mineral N or small letter for reserves N are not significantly different (P ≤ 0.05, n = 5).

Fig. 5. Comparison between exogenous N translocation (left, calculated from Eq. (2)) and endogenous N remobilization (right, calculated from Eq. (4)) in +P or −P plants over 24 days of regrowth. Each value (mg N per plant) is the mean ± S.E. for n = 5.
shoot (leaves + stubble) dry weight, and thus a substantial decline in the shoot-to-root dry weight ratio with longer P deficiency (Table 1). The reduction in the development of new foliage in -P plants was mainly due to an effect on the expansion of individual leaves. Leaf expansion has been shown to be positively correlated with leaf epidermal cell area as the P status of cotton leaves is increased (Radin and Eidenbock, 1984). P concentrations in the upper epidermis are sharply reduced with decreased P supply to the leaf (Treeby et al., 1987). Fibrous root growth was increased by P deprivation (Table 1). One possible explanation for this is that -P plants diverted more of their photosynthate toward roots because less was utilized in shoot growth (Fredeen et al., 1989). Certainly P withdrawal from the nutrient medium caused to reduce P content in whole plant part (Fig. 1) and it was accompanied by a decrease in new foliage development and an increase in dry weight of roots (Table 1).

We tentatively hypothesized that 7 days of external P deprivation for the 15N pulse feeding period before defoliation would lead to significant changes in the 15N partitioning and in consequence the pool size of N compound in remaining organs. This appeared to be true, as is shown in Fig. 2. A large part of 15N was found in stubble at day 0 (cutting date), accounting for 2–4 times higher amount than roots regardless of P treatments. This indicates that the stubble remaining after defoliation is a primary storage site for N compounds, in accordance with the finding of Ourry et al. (1988) and Morvan-Bertrand et al. (1999) who showed in perennial ryegrass a high accumulation of organic N compounds before defoliation and an active mobilization during regrowth. The data for 15N amount at day 0 (Fig. 2) provide two notable responses to P deprivation before defoliation: (i) the accumulation of all N compounds in main reserve organ (stubble) and (ii) an accumulation of amino acids in roots. These clearly indicate that N utilization was being disrupted in -P plants. Rufty et al. (1993) have shown in NO3-fed soybean plants that nitrate accumulated in roots and soluble-reduced N accumulated in all plant parts when P availability was low. These observations lead us to suggest that N utilization at whole plant level and translocation of reduced N to aerial parts are restricted by P deficiency before defoliation.

15N tracing during regrowth demonstrated that N reserves labeled in stubble and roots before cutting continuously decreased (Fig. 2), whereas 15N-labeled nitrogenous compound in the regrowing shoots increased throughout experiment (Fig. 3) clearly indicating N reserves mobilization into regrowing leaves. In both +P and -P plants, the largest decline of 15N in remaining organ occurred for the first 6 days (Fig. 2), indicating a higher utilization of N reserves. The N supply for regrowth appears to be highly dependent on N reserves during an early phase of regrowth as in perennial ryegrass (Ourry et al., 1988; Louahlia et al., 1999) and alfalfa (Kim et al., 1991; Volene et al., 1996). It was noticeable that the decreasing rate of 15N for the first 6 days in most N compounds in remaining organ (except for proteins in roots) was much higher in -P plants (Fig. 2). This clearly indicated that the pool size of N reserve compounds in remaining organs was more important in -P plants than in +P plants to initiate the regrowth.

A higher utilization of N reserves in -P plants for the early regrowth might be associated with a compensatory increase to a lower uptake of mineral N under P-deprived condition. In fact, 14N uptake from nutrient solution for the first 6 days were 19.5 and 14.6 mg per plant in P-sufficient and P-deprived condition, respectively (data not shown). The possibility that 14NO3 uptake was restricted by effects associated with the higher amount of NO3 and amino acids in stubble and roots of -P plants at the beginning of regrowth (Fig. 2) appears particularly plausible. Both have been implicated in feedback control of the uptake process. Negative correlation between NO3 concentration in root tissue and NO3 uptake rate has been observed in a number of experiments (Lee and Rudge, 1986; Siddiqi et al., 1990). It has been proposed previously that root amino acid levels function as regulatory intermediates in the feedback control system coordinating shoot demand for N and NO3 uptake by roots (Cooper and Clarkson, 1989; Rufty et al., 1993). In our experiment, it could be suggested...
that any of nitrate and amino acids previously accumulated in stubble have been involved in the regulation of external N uptake. A stimulation for the utilization of N reserves in —P plants during the first 6 days of regrowth could be explained by a higher availability of N reserves in —P plants than +P plants, in accordance with results of Oury et al. (1994).

The results demonstrate that a stimulation of N reserves utilization caused by P deprivation for the first 6 days gradually disappears from day 12, showing the lags of 15N increase in regrowing leaves (Fig. 3). Furthermore, contribution of reserves N for N increment in regrowing leaves begins to be restricted by P deprivation and the newly absorbed N becomes a predominant source for shoot regrowth from this period (Fig. 4). At day 24, more than 70% of total shoot N was derived from mineral uptake for both P-treated plants. In several species, such as *Medicago sativa* (Kim et al., 1991; Avice et al., 1996; Volene et al., 1996) or *L. perenne* (Oury et al., 1988; Louahlia et al., 1999), it has been shown that newly absorbed N much actively contribute to meet N demand for regrowth when new foliage has been developed largely enough. The data obtained indicate that mineral N uptake and further translocation to the regrowing leaves are more and more restricted (Fig. 4), matching with noticeable decline in P concentration in stubble and roots (Fig. 1) as regrowth proceeds on —P medium.

Total P and P_i concentration in all three organs greatly decreased in —P plants in accordance with the results obtained from soybean (Fredeen et al., 1989) and bean roots (Rychter and Randall, 1994). The difference in P_i concentration between +P and —P plants was gradually increased as regrowth progressed (Fig. 1). It was suggested that internal P_i availability was highly decreased in —P plants at later period of regrowth. This consequently limits the phosphorylation of sugars. Rychter and Randall (1994) reported that in P-deficient bean roots the ratio of [glucose-6-P + fructose-6-P] to [glucose-6-P + fructose-6-P] was about 7.2 after 17 days of treatment, while in P-sufficient roots this ratio was 1.4. These results indicate that the pools of non-phosphorylated and phosphorylated sugars were not equilibrated in P-stressed plants contrary to the P-sufficient plants where this ratio was close to 1. Furthermore, the lower P_i availability in —P plants might cause to decrease the size of ATP pool, considering the results of Rychter and Randall (1994) who reported that a marked decrease in ATP pool was observed after prolonged P deficiency. Thus, low ATP as a consequence of low P_i availability might be expected to restrict active mineral N uptake against an electrochemical potential gradient or, more probably, to limit synthesis of membrane transport system for mineral N (especially for NO_3^-), which was indicated in previous kinetic experiments with P-stressed barley (Rufy et al., 1991). As shown in a summary diagram of N flow over 24 days of regrowth (Fig. 5), the considerably low N increment in the regrowing leaves of —P plants is principally due to decreased mineral N uptake and to restricted remobilization of N reserves as well.

In conclusion, for the early regrowth, an extensive utilization of N reserves in —P plants occurs to compensate a lower uptake of external N as a kind of adaptive response to P-limited stress. At later regrowth period (regrowth days 12–24 in this experiment) when shoot regrowth proceeds rapidly, an inhibitory effect of P deficiency on N utilization for both N reserves and mineral N appears to be distinct, so that a negative response of shoot regrowth follows to P deficiency.

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