Plant, Cell and Environment (1989) **12**, 407–418

Relationships between nitrogen uptake and carbon assimilation in whole plants of tall fescue

F. GASTAL[1] & B. SAUGIER[2] [1]Laboratoire d'Ecophysiologie des plantes fourragères Institut National de la Recherche Agromomique, 86600 Lusignan, and [2]Laboratoire d'Ecologie Végétale (URA CNRS N° 14), Université Paris-Sud, Bâtiment 362, 91405 Orsay Cedex, France

Received 4 January 1987; Received in revised form 18 October 1988; accepted for publication 2 November 1988

Abstract. The present study investigates the relationships between nitrogen uptake, transpiration, and carbon assimilation. Plants growing on nutrient solution were enclosed for 10–16 d in a growth chamber, where temperature, photon flux density, vapour saturation deficit and CO_2 concentration were controlled. One of these factors was modified every 4 to 5 d. Shoot photosynthesis and root and shoot respiration were recorded every half-hour. Nitrogen uptake from the root medium and plant transpiration were measured daily. In most cases, an increase in photon flux density led to increases in transpiration, net daily carbon assimilation, and nitrogen uptake. By modifying transpiration rate without changing photosynthesis (varying vapour saturation deficit), or by modifying transpiration and carbon assimilation in opposite ways (varying CO_2 air concentration), it was shown that nitrogen uptake does not follow transpiration, but is linked to the carbon uptake of the plant. When light was increased from low to intermediate levels, the N uptake/C assimilation ratio remained constant. At higher photon flux density, this ratio declined markedly. It is proposed that in the first case, growth is limited by carbohydrate availability, thus any increase in carbon assimilation leads to a proportional increase in nitrogen uptake, in contrast to the second situation where carbohydrates may accumulate in the plant without further nitrogen requirement.

Key-words: light; air humidity; CO_2 concentration; transpiration.

Introduction

In field experiments, nitrogen uptake is generally evaluated from nitrogen content and biomass of plants harvested on weekly intervals. It has been shown to be mainly determined by the growth rate, when the soil is well supplied with nitrogen (Greenwood, 1978; Lemaire, 1984). Laboratory studies lead to similar conclusions and relative growth rate of a plant is proportional to the relative rate of nitrogen supply in the environment (Ingestad, 1982).

Physiological approaches have enlightened the dependency between nitrate absorption and nitrate assimilation (Huffaker & Rains, 1978), between nitrate flow in the plant and nitrate reduction (Shaner & Boyer, 1976). However, little is known about the integrated control of nitrogen uptake and utilization at the whole plant level.

Data concerning the effect of transpiration on nitrogen uptake in intact plants are rather confusing, but it seems unlikely that nitrogen uptake is controlled by root water uptake.

With recent advances in phytotronics, it became possible to study the effects of climatic factors on nitrogen uptake, on a hourly or daily time step. Such works have revealed diurnal fluctuations in the rate of nitrate uptake (Pearson & Steer, 1977; Triboi, 1979; Hansen, 1980) in phase with variations in light level and in the rate of photosynthesis (Clement, Hopper & Jones, 1978b; Massimino *et al.*, 1981). However, these studies have often failed to relate precisely growth with N uptake rates. The aim of the present study was to investigate such relationships on a daily basis.

Material and methods

Tall fescue seedlings (*Festuca arundinacea* L., cult. Clarine) were grown in a controlled room on nutrient solution. Photoperiod was 14 h, photosynthetic photon flux density (PPFD) was 440 μmol $m^{-2} s^{-1}$, temperature and relative air humidity were held constant at respectively 20 °C and 70%. Nutrient concentration in root medium were: 1.90 mol m^{-3} KNO_3, 0.55 mol m^{-3} $Ca(NO_3)_2$, 2.50 mol m^{-3} NO_3NH_4, 0.50 mol m^{-3} $CaCl_2$, 0.10 mol m^{-3} NaCl, 0.50 mol m^{-3} $MgSO_4$, 0.40 mol m^{-3} PO_4H_2K, 0.30 mol m^{-3} PO_4HK_2 + micro-elements.

After 4–6 weeks of growth, a single plant with a number of tillers was transferred into a chamber for measurements of CO_2 and H_2O exchanges and of nitrogen uptake. In the chamber, temperature was maintained at 24 ± 1.5 °C by a heating/cooling system. Shoots and roots were enclosed in two distinct compartments, allowing separate measurements (Fig. 1). Both compartments were closed, and the regulation of gas concentration was achieved by automatic addition or removal of given amounts of the gases. This allowed maintenance of concentrations at preset values and measurements of exchanges with the atmosphere.

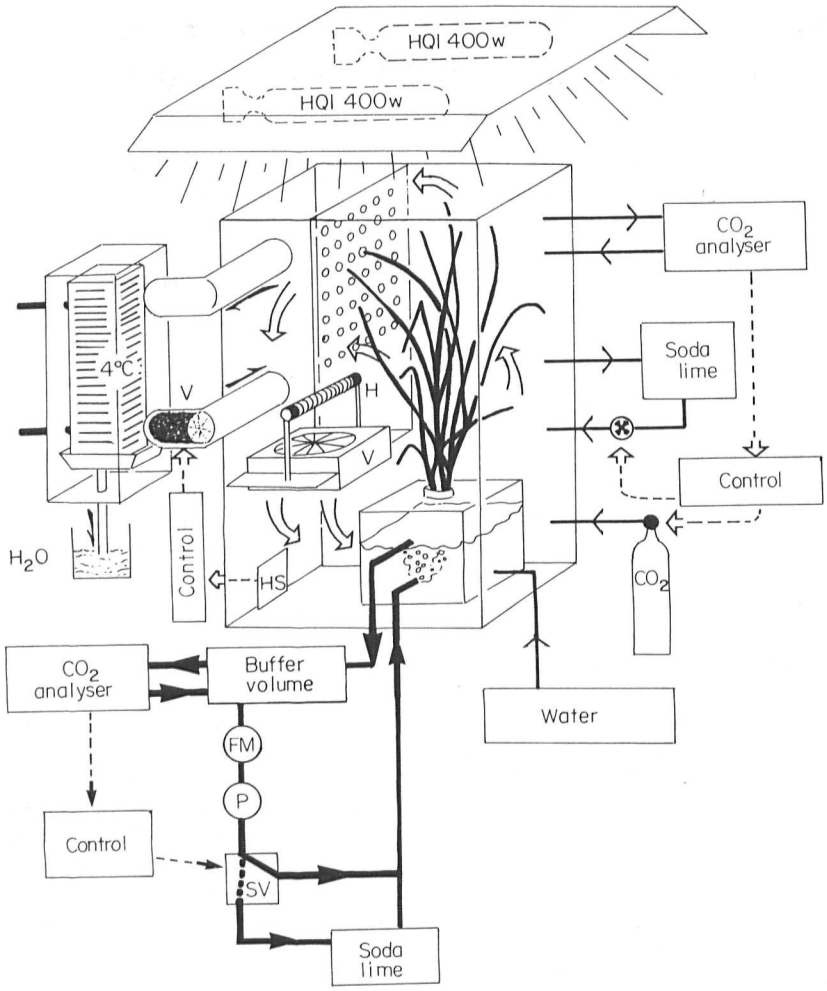

Figure 1. General view of the apparatus for gas exchanges and nitrogen uptake measurements. V: fan; H: heating unit; HS: air humidity sensor; FM: flow meter; P: air pump; SV: tri-way solenoid valve.

Photosynthesis, respiration and transpiration of shoots

Maximum PPFD was 875 μmol m^{-2} s^{-1} and could be decreased with screens.

In the shoot compartment, the totality of the air was passed through the fan every 3–5 s.

Air CO_2 concentration was held constant either by injection of a known quantity of an air mixture (CO_2 5%–N_2 95%) during periods of photosynthesis, or by passing a known flow rate of the air over soda lime for a given time during periods of respiration. Photosynthesis or respiration was measured over 30-min periods by counting, respectively, the number of injections achieved and the number of times the by-pass to soda-lime was switched on. Carbon dioxide concentration was continuously measured with an infra-red gas analyser.

Vapour saturation deficit was measured by a capacitance sensor, and maintained constant ($\pm$8%) by the discontinuous functioning of a mini-fan which passed a fraction of the air through an external condenser column held at 4 °C, and returned it to the enclosure. Transpiration was measured over 24 h as the quantity of water collected at the bottom of the condenser.

Root respiration

The root compartment was enclosed within the shoot compartment (Fig. 1). It contained nutrient solution and a gaseous phase at the top, from which air was pumped to an external buffer volume and then returned to the base of the vessel through an aerating system.

The carbon dioxide concentration of the air in the buffer volume was continuously monitored and regulated (330 ± 20 μmol mol^{-1}) by by-passing when necessary the air returned to the root cuvette over soda-lime. The flow-rate was constant during a day and adjusted according to the size of the root system. Root respiration was evaluated by determining the total time this air-flow was bypassed over the soda-lime during a 30-min period.

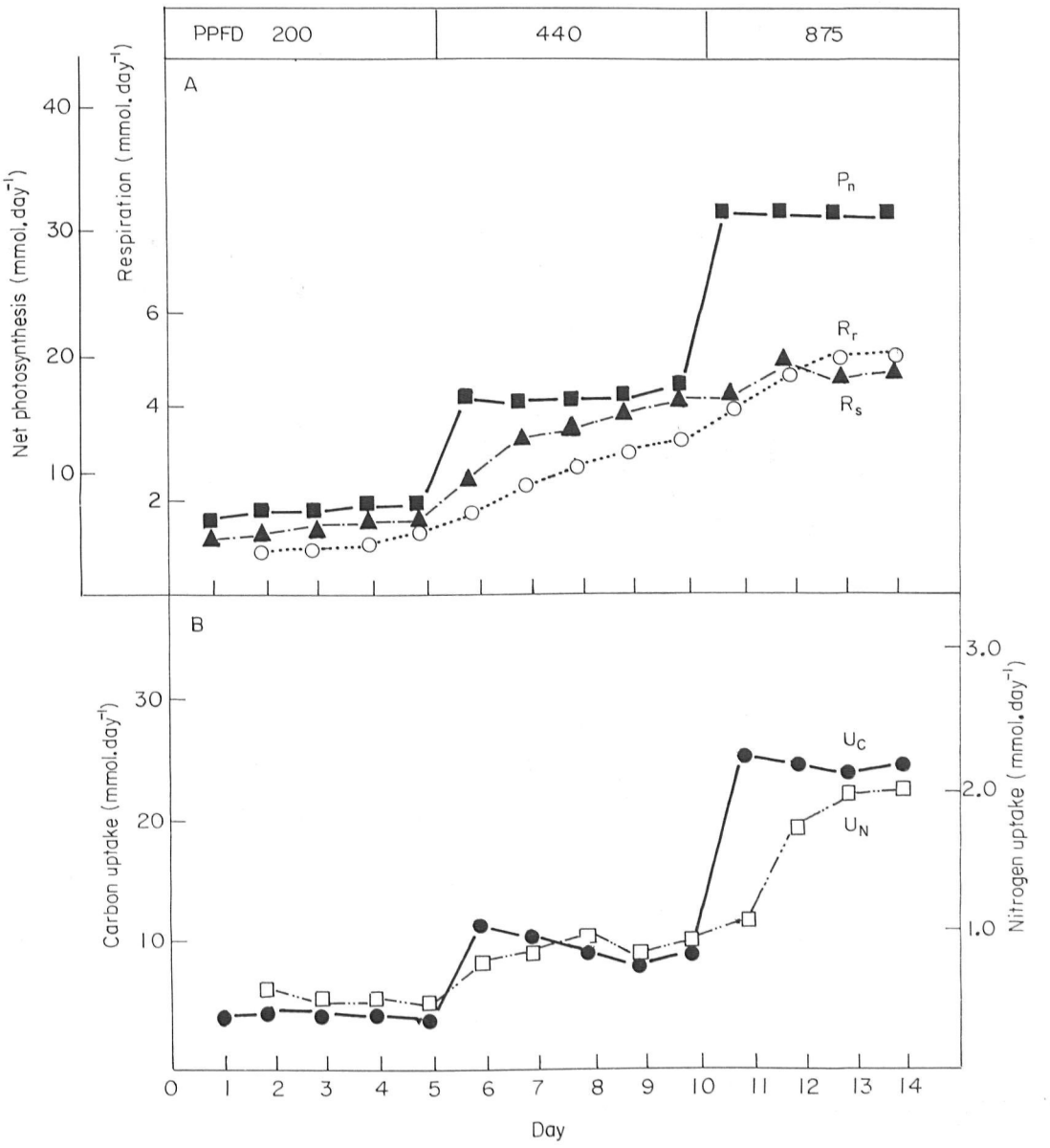

Figure 2. Daily variations of net photosynthesis P_n (■), shoot respiration R_s (▲), root respiration R_r (○), carbon uptake U_C (●) and nitrogen uptake U_N (□), for experiment 1. Constant CO_2 concentration (330 μmol mol^{-1}), vapour saturation deficit (1.35 kPa). PPFD was raised from 200 to 440 and 875 μmol m^{-2}s^{-1}.

Daily net CO_2 uptake

The daily net CO_2 uptake was computed by integrating over 24 h the half-hourly measurements of net photosynthesis, shoot respiration and root respiration (respectively P_n, R_s, R_r). The daily net Carbon uptake (U_C) was then determined as:
$U_C = P_n - R_s - R_r$ (mmol d^{-1})

Nitrogen uptake

Water uptake was compensated for by an automatic system that added distilled water to the root vessel to maintain a constant level of nutrient solution.

The composition of the root medium was the same as the one used during pre-culture (5.5 mol m^{-3} NO_3^-, 2.5 mol m^{-3} NH_4^+). The pH varied in the range 5.5–6.0.

Each day at the same hour, the enclosure was opened, and the nutrient solution was completely renewed. The nitrate and ammonium concentrations of the new solution and of the solution after 24 h of contact with the roots were determined. Nitrate concentration was measured after transformation into nitrite using the colorimetric method of Cataldo *et al.* (1975). Ammonium concentration was measured with a specific ion electrode.

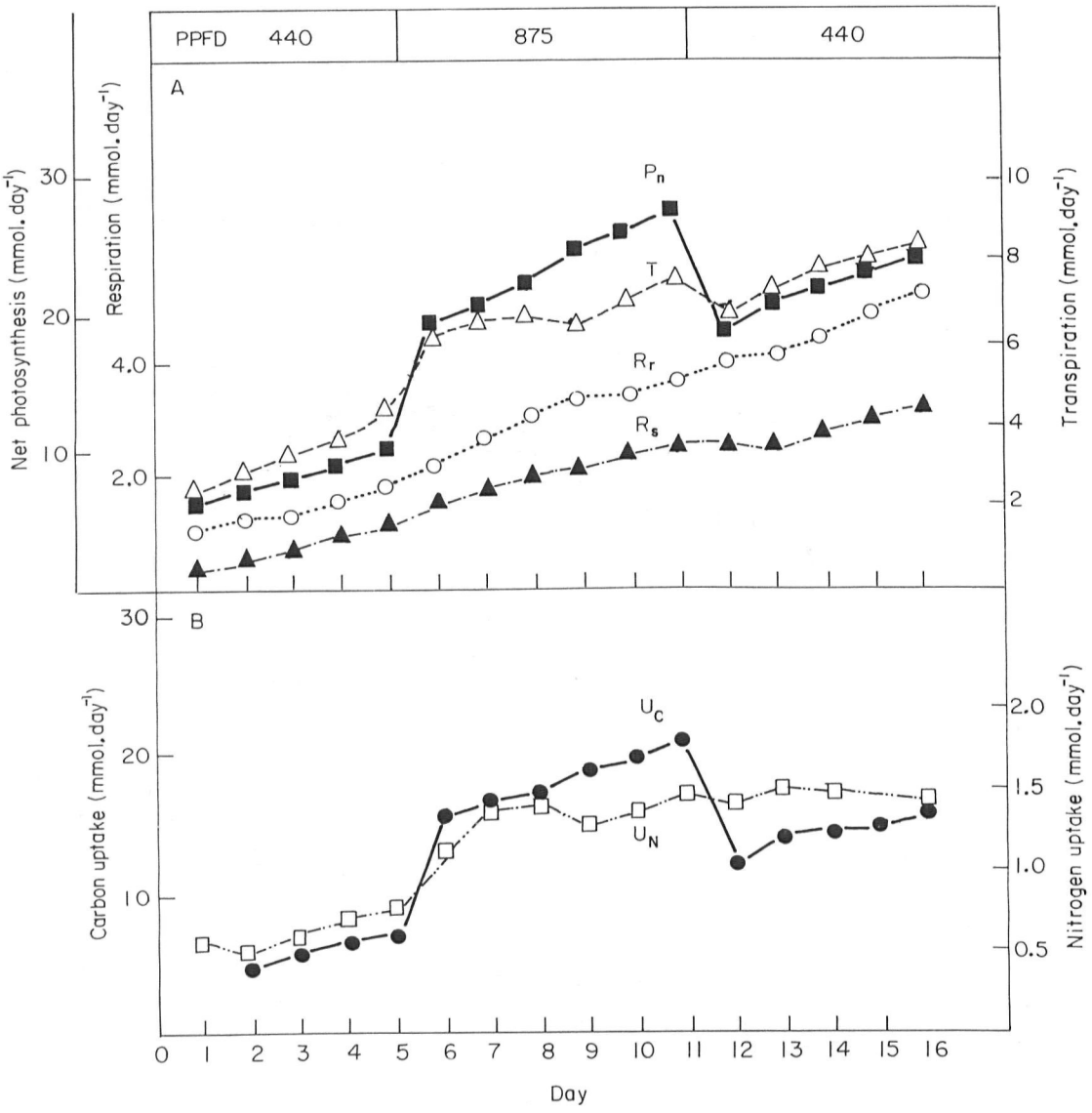

Figure 3. Experiment 2. Symbols as in Fig. 3 but include variations of transpiration T (△). Constant CO_2 concentration (330 μmol mol^{-1}) and vapour saturation deficit (1.35 kPa). PPFD changed from 440 to 875 and 440 μmol m^{-2}s^{-1}.

Table 1. Environmental conditions for the seven experiments. L is photosynthetic photon flux density in μmol m^{-2} s^{-1}, CO_2 is the air CO_2 concentration in μmol mol^{-1}. V.S.D. is the vapour saturation deficit in kPa

	L	CO_2	V.S.D.		L	CO_2	V.S.D.
Experiment 1a	200	330	1.35	Experiment 2a	440	330	1.35
b	440	330	1.35	b	875	330	1.35
c	875	330	1.35	c	440	330	1.35
Experiment 3a	440	330	1.35	Experiment 4a	875	330	1.35
b	875	330	1.35	b	875	330	0.30
c	200	330	1.35				
Experiment 5a	200	330	1.35	Experiment 6a	875	330	1.35
b	875	330	1.35	b	875	660	1.35
c	875	660	1.35				
Experiment 7a	440	330	1.35				
b	875	330	1.35				
c	875	660	1.35				

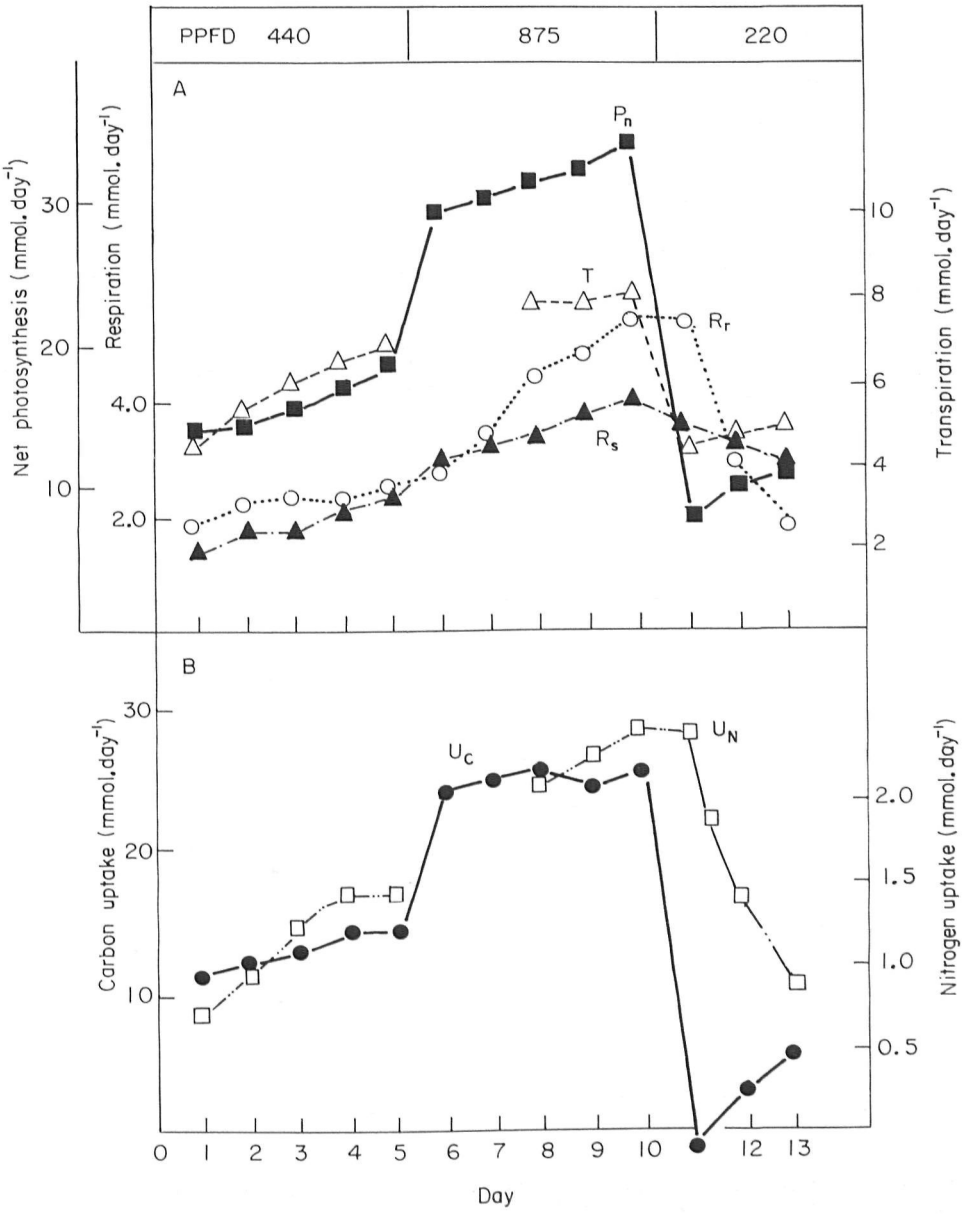

Figure 4. Experiment 3. Symbols as in Fig. 3. Constant CO_2 concentration (330 μmol mol^{-1}) and vapour saturation (1.35 kPa). PPFD changed from 440 to 875 and 220 μmol m^{-2}s^{-1}.

In all cases, NO_3^- and NH_4^+ concentrations in the root medium remained above 2 mol m^{-3} and 0.7 mol m^{-3}, respectively, during the 24 h of contact with the roots. In this range, concentrations do not affect substantially the rate of uptake (Lycklama, 1963; Clement, Hopper & Jones, 1978a; Ingestad, 1982).

The daily nitrogen uptake of the plants (U_N) was calculated by:

$$U_N = V_1.N_1 - V_2.N_2$$

where N_1 and N_2 are the initial and final nitrogen concentration ($NH_4^+ + NO_3^-$) of nutrient solution and V_1 and V_2 are the initial and final volumes, respectively. Normally these volumes are the same since transpired water is automatically replaced.

Experimental procedure

Seven experiments were conducted, all of them in a similar way. A sward, previously grown in the culture room, was installed in the measurement enclosure. Gas exchanges and nitrogen uptake measurements were started. Environmental conditions (air CO_2 concentration, vapour saturation deficit, PPFD) were kept constant for 4–5 d (initial conditions a). One factor was then modified and maintained constant

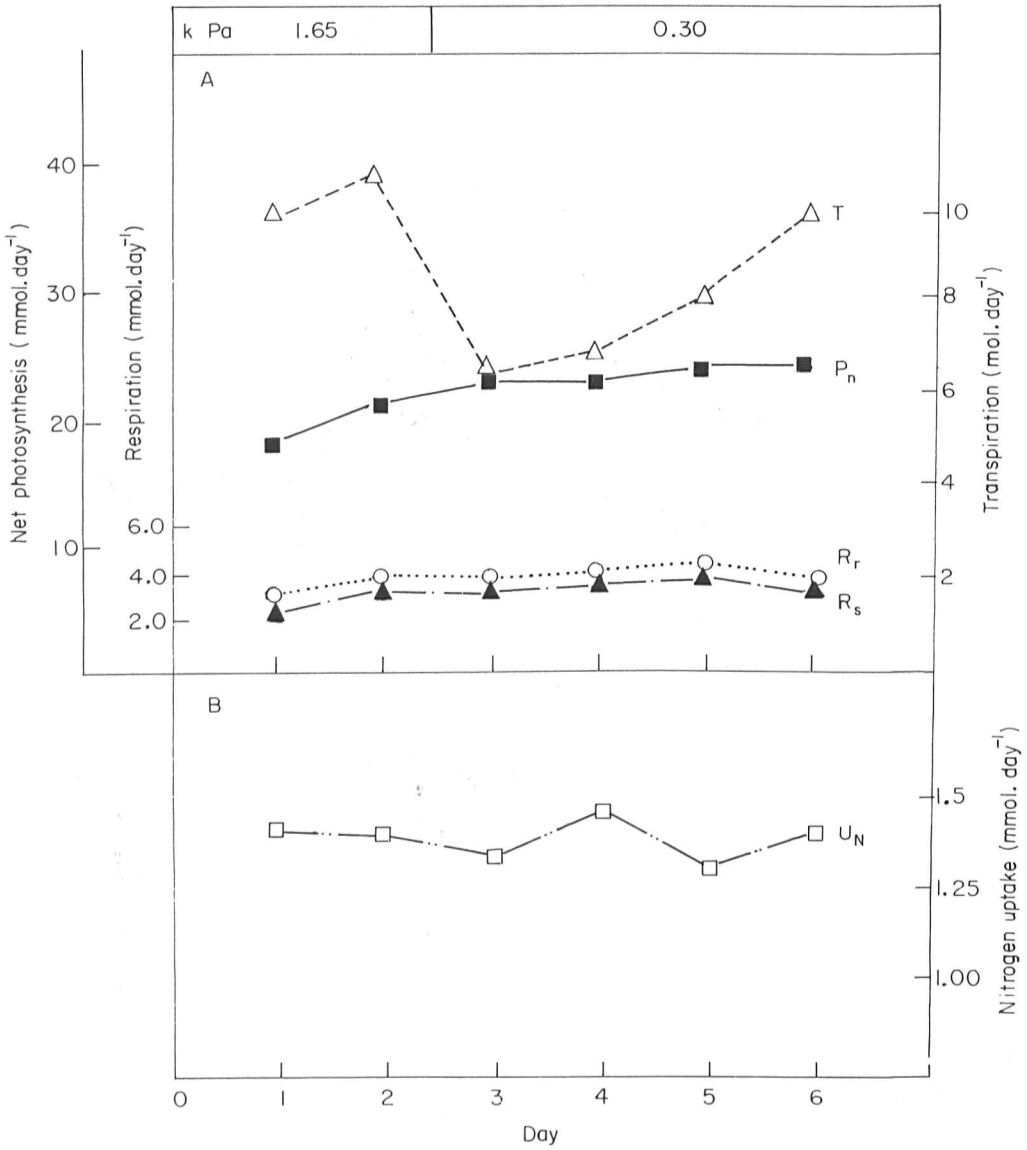

Figure 5. Experiment 4. Symbols as in Fig. 3. Constant CO_2 concentration (330 μmol mol^{-1}) and PPFD (875 μmol m^{-2}s^{-1}). Vapour saturation deficit changed from 1.65 to 0.30 kPa.

for a new 5-d period (conditions b). Then the same factor—or another one—was again changed (conditions c), and the measurements lasted 3–4 more days.

Details of the environmental conditions imposed during the seven experiments are given in Table 1.

Results

The daily values of photosynthesis, and shoot and root respiration for experiments 1 to 3 are presented in Figs 2A to 4A. Net daily carbon and nitrogen uptakes are shown in Figs 2B to 4B. For these experiments and most of the other ones, all the measured parameters increased regularly each day under steady state conditions of environment (see for instance Fig. 3). This increase resulted from the growth of shoots and roots during the course of the experiment.

When a change in environmental conditions was induced (i.e. the rise in PPFD at day 6, Fig. 3), photosynthesis and transpiration showed an immediate response, and a new rate of increase of these parameters was reached the following day. For respiration rates and nitrogen uptake, the response was less rapid and a transitory state was superposed to the more regular increase observed during the previous steady state conditions of environment. This lag phase lasted generally 2 to 3 d after the change in PPFD.

Experiments 1 to 3 followed the same pattern of response to variation in PPFD. In all cases, a rise in

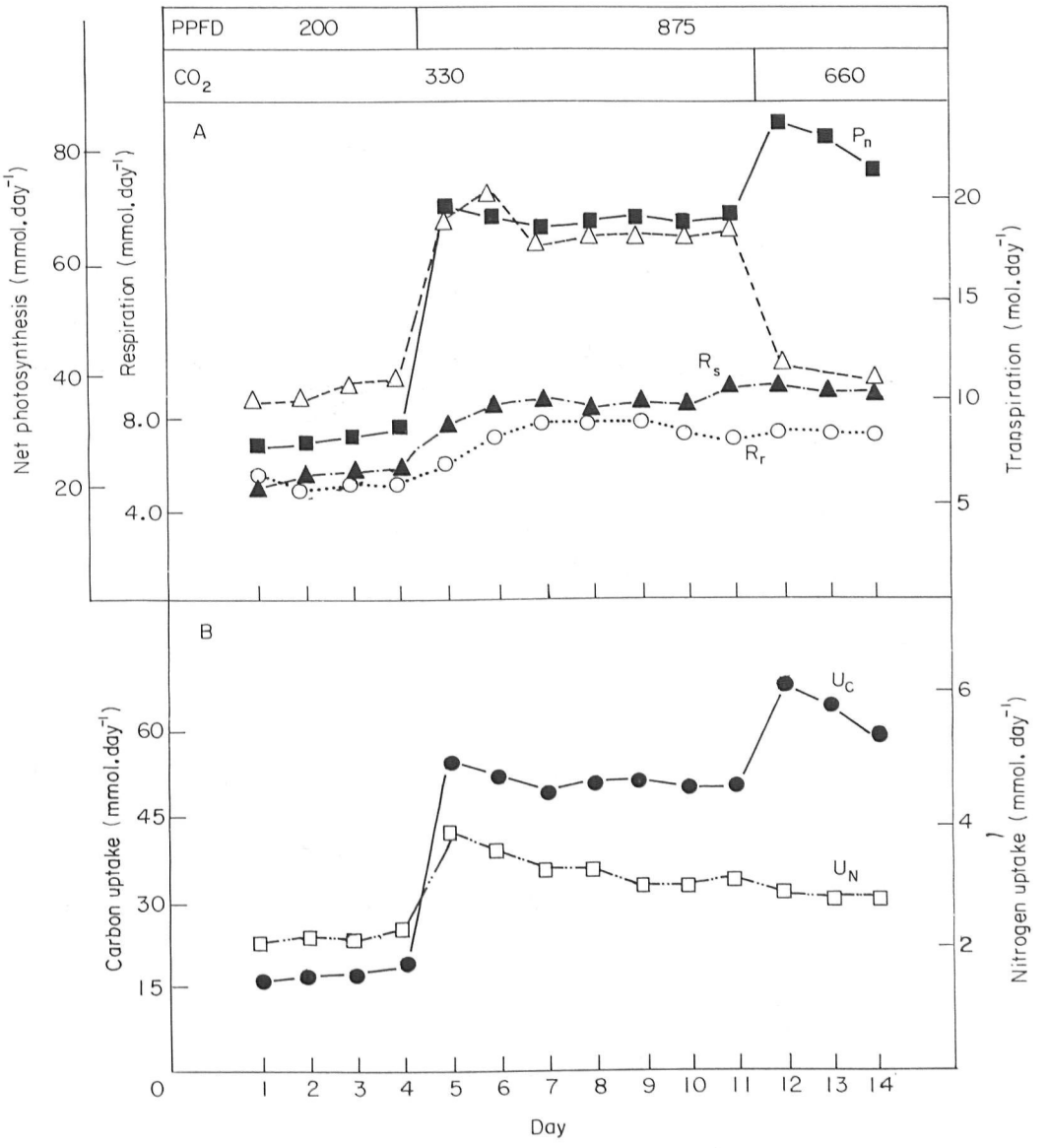

Figure 6. Experiment 5. Symbols as in Fig. 3. Constant vapour saturation deficit (1.35 kPa). PPFD changed from 200 to 875 μmol m^{-2}s^{-1} and CO_2 concentration from 330 to 660 μmol mol^{-1}.

PPFD led to a stimulation of transpiration, carbon assimilation, and nitrogen uptake. Conversely, a decline in PPFD was followed by a decline in these three parameters.

In the next experiments, the effects of transpiration on nitrogen were separated from those of photosynthesis using two different experimental approaches.

In the first, vapour saturation deficit was decreased from 1.65 to 0.30 kPa (Exp. 4, Fig. 5), and this led to a 45% decrease in transpiration rate, with a trend to a partial recovery during the following days. Photosynthesis and respiration rates remained unchanged, as well as nitrogen uptake.

In the second series, CO_2 air concentration was raised from 330 to 660 μmol mol^{-1} at the high PPFD (experiments 5, 6 and 7). In all cases (Figs 6 & 7), the authors observed a stimulation of photosynthesis (+30%) and a marked decline of transpiration (−35%). Nitrogen uptake showed no change or a slight increase (Figs 6 & 7 and experiment 7 not shown).

In no instance did the reduction of transpiration induced by CO_2 enrichment led to a fall in nitrogen uptake. The same conclusion was reached when vapour saturation deficit of air was changed: nitrogen uptake and transpiration of the plant behaved independently, at least in these conditions where there was no shortage of nitrogen or water.

The ratio of NO^-_3 uptake to (NO^-_3+NH^+_4)

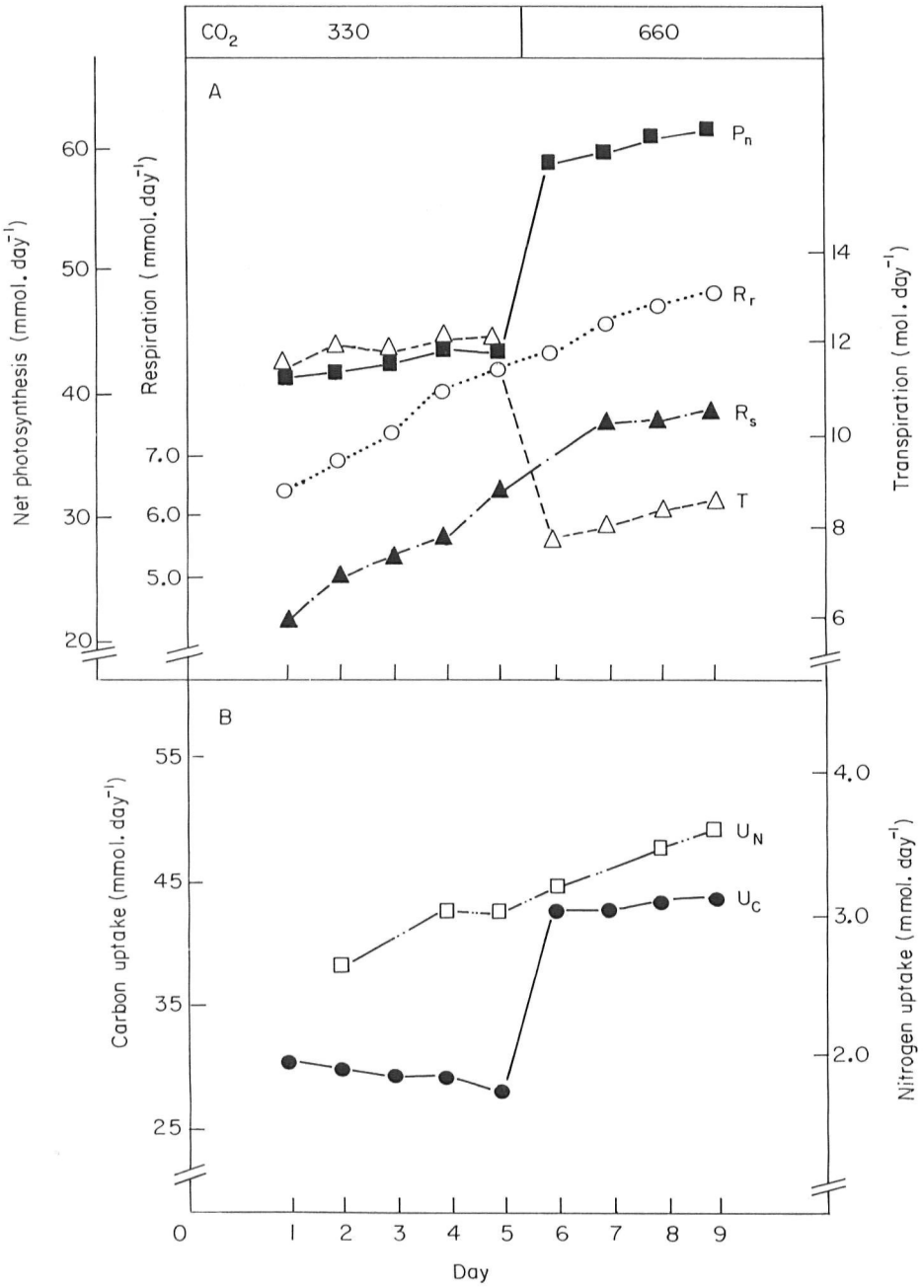

Figure 7. Experiment 6. Symbols as in Fig. 3. Constant PPFD (875 μmol $m^{-2}s^{-1}$) and vapour saturation deficit (1.35 kPa). CO_2 concentration raised from 8330 to 660 μmol mol^{-1}.

uptake generally did not vary much when either light level or CO_2 air concentration was modified (results not shown), and stayed between 0.65 and 0.55 depending on the experiment. However, in experiments 1 and 4, this ratio tended to decrease when PPFD level was increased.

Discussion

All the experiments were conducted under steady state, non-limiting conditions of nitrogen availability in the root medium, as during pre-culture. These conditions are known to lead to high concentrations of nitrate in leaf tissues. Previous experiments have shown that when growth conditions remain unchanged, the NO_3^- content in tall fescue is constant during the entire growth period (Gastal & Saugier, 1986), although there are slight diurnal fluctuations (Clement *et al.*, 1978b).

The molar fraction of absorbed nitrogen to assimilated carbon was close to 0.12 at an intermediate photon flux density (Fig. 8). This is

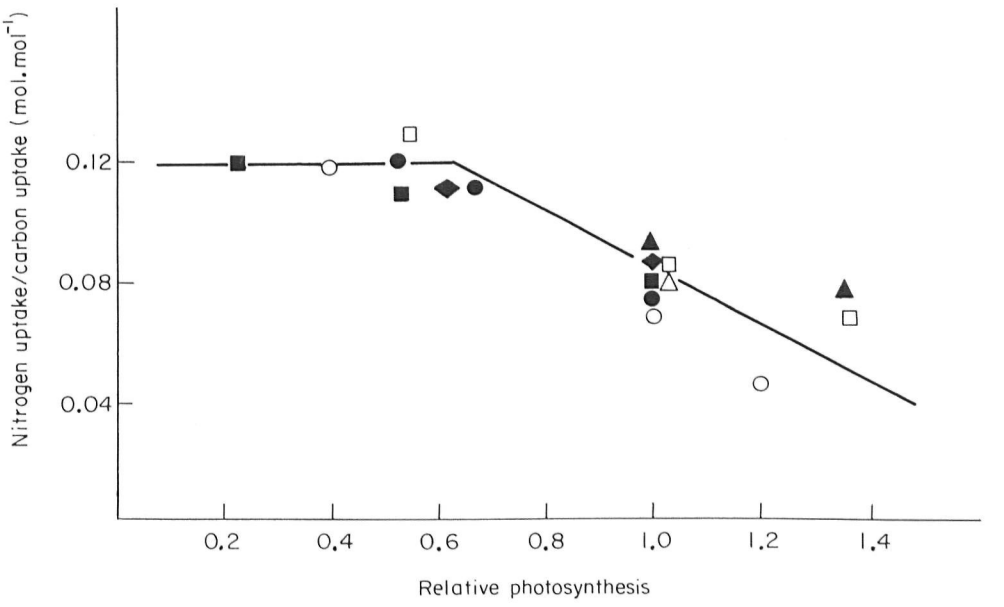

Figure 8. Relationship between relative photosynthesis and the ratio between daily nitrogen uptake and daily carbon assimilation. Each symbol refers to a different experiment (see table I): ■ 1, ● 2, ◆ 3, △ 4, ○ 5, ▲ 6 and □ 7.

equivalent to a nitrogen content of 5.6 with respect to dry matter, assuming a carbon content of 40%. Such a value is commonly observed for plants which accumulate nitrate. The ratio of shoot dark respiration + root daily respiration to daily photosynthesis varied in the range 25–40%, an order of magnitude frequently found in other studies. The relative growth rate was estimated from the net carbon assimilation over the last 24 h for each experiment with an assumption of 0.4 g carbon per g biomass for daily dry matter increment, and biomass measured at the end of the experiment. The values obtained varied in the range 0.08–0.12 $g.g^{-1} d^{-1}$ at the highest PPFD. These points confirm the coherence of the measurements reported here.

After a change in CO_2 concentration, in PPFD, or in vapour saturation deficit, stabilization of nitrogen uptake generally took over 1 or 2 d longer than the change in carbon uptake or in transpiration. Since only daily values are reported here, this period of adjustment is fully compatible with data from Clement (1978b), who reported a time lag of about 6 h.

These results show clearly the independence of water and nitrogen uptake (experiments 3, 4 and 6) as previously reported by Schulze & Bloom (1984), and in agreement with data showing that the ratio of nitrate uptake to water uptake varies greatly between day and night (Triboi, 1979; Massimino *et al.*, 1981).

To investigate the relationships between nitrogen and carbon uptake, the present authors have computed the ratio of nitrogen uptake to carbon uptake during steady states. To do this, they have used mean data of the last 2 or 3 d following 2 d of transient state after a change in conditions, or after installation of the plant in the chamber. These data have been plotted against photosynthesis normalized with respect to its value at the maximal photon flux density (875 $\mu mol\, m^{-2} s^{-1}$) and normal CO_2 concentration (330 $\mu mol\, mol^{-1}$), to allow a comparison between plants of various sizes (Fig. 8).

This ratio remained constant at PPFD of 200 and 440 $\mu mol\, m^{-2} s^{-1}$. At higher PPFD and/or higher CO_2 levels, it decreased when photosynthesis increased.

These results show that:

(1) daily nitrogen uptake is proportional to net daily carbon assimilation when PPFD is low or intermediate. If we consider that growth is proportional to net daily carbon assimilation, and biomass does not vary substantially within a few days, these results imply a proportional relationship between nitrogen uptake and relative growth rate when PPFD stayed in this range. They are in close agreement with results of Ingestad & Ågren (1988).

(2) for higher PPFD, nitrogen uptake seems to reach a limit, which might be determined by nitrogen assimilation capacity of the plant or by its maximal growth rate. In this case, there is no more proportionality between nitrogen uptake and relative growth rate.

These data at high PPFD are not in accordance with the hypothesis of Raper *et al.* (1978), who postulated that N uptake was related to the level of soluble carbohydrates reaching the roots, as in the authors' experiments any increase in photon flux density should lead to an increase of C translocation with respect to C assimilation (Ryle & Powell, 1976).

The results presented here confirm the hypothesis that synthesis of new living tissues is limited by carbohydrate availability, up to intermediate PPFD. In these conditions of PPFD, any stimulation of carbon supply leads to a proportional increase in nitrogen demand for synthesis of living material with a more or less constant C/N ratio. At higher PPFD, a part of the carbon assimilated may be stored as reserve carbohydrates, which do not lead to substantial requirement for nitrogen.

Acknowledgments

We are grateful to Dr M. L. Champigny for her criticism of the manuscript.

References

Cataldo, D.A., Haroon, M., Schrader, L.E. & Youngs, V.L. (1975) Rapid colorimetric determination of nitrate in plant tissue by nitratation of salicylic acid. *Soil Science and Plant Analysis*, **6**, 71–80.

Clement, C.R., Hopper, M.J. & Jones, L.H.P. (1978a) The uptake of nitrate by *Lolium perenne* in flowing nutrient solution. I. Effect of NO_3 concentration. *Journal of Experimental Botany*, **29**, 453–464.

Clement, C.R., Hopper, M.J. & Jones, L.H.P. (1978b) The uptake of nitrate by *Lolium perenne* in flowing nutrient solution. II. Effect of light, defoliation, and relationship to CO_2 flux. *Journal of Experimental Botany*, **29**, 1173–1183.

Gastal, F. & Saugier, B. (1986) Alimentation azotée et croissance de la fétuque élevée. II. Absorption de l'azote et distribution dans la plante. *Agronomie*, **6**, 363–370.

Greenwood, D.J. (1978) A theoritical model for the decline in the protein content in plants during growth. *Journal of Agricultural Science, Cambridge*, **91**, 461–466.

Hansen, G.K. (1980) Diurnal variation of root respiration rates and nitrate uptake as influenced by nitrogen supply. *Physiologia Plantarum*, **48**, 421–427.

Huffaker, R.C. & Rains, D.W. (1978) Factors influencing nitrate acquisition by plants; assimilation and fate of reduced nitrogen. In *Nitrogen and the environment*, Vol. 2 (eds D. R. Nielsen & J. G. MacDonalds), pp. 1–43. Academic Press, London.

Ingestad, T. (1982) Relative addition rate and external concentration; driving variables used in plant nutrition research. *Plant, Cell and Environment*, **5**, 443–453.

Ingestad, T. & Ågren, G.I. (1988) Nutrient uptake and allocation at steady state nutrition. *Physiologia Plantarum*, **72**, 450–459.

Lemaire, G. (1984) Relation entre dynamique de croissance et dynamique de prélèvement d'azote pour un peuplement de graminées fourragères. I. Etude de l'effet du milieu. *Agronomie*, **4**, 423–430.

Lycklama, J.C. (1963) The absorption of ammonium and nitrate by perennial rye-grass. *Acta Botanica Neerlandica*, **12**, 361–423.

Massimino, D., André, M., Richaud, C., Daguenet, A., Massimino, J. & Vivoli, J. (1981) The effect of a day at low irradiance of a maize crop. I. Root respiration and uptake of N, P, K. *Physiologia Plantarum*, **51**, 150–155.

Pearson, C.J. & Steer, B.T. (1977) Daily changes in nitrate uptake and metabolism in *Capsicum annuum*. *Planta*, **137**, 107–112.

Raper, C.D., Osmond, D.L., Wann, L. & Weeks, W.W. (1978) Interdependence of root and shoot activity in determining nitrogen uptake rate of roots. *Botanical Gazette*, **139**, 289–294.

Ryle, G.J.A. & Powell, C.E. (1976) Effect of rate of photosynthesis on the pattern of assimilate distribution in the gramineous plants. *Journal of Experimental Botany*, **27**, 189–199.

Schulze, E.D. & Bloom, A.J. (1984) Relationship between mineral nitrogen influx and transpiration in radish and tomato. *Plant Physiology*, **76**, 827–828.

Shaner, D.L. & Boyer, J.S. (1976) Nitrate reductase activity in maize leaves. I. Regulation by nitrate flux. *Plant Physiology*, **58**, 499–504.

Triboi, A.M. (1979) Dynamique comparée de l'absorption des nitrates et de l'eau par des plantules de blé. *Comptes Rendus des Séances de l'Académie des Sciences, Paris*, **288**, série D, 1545–1548.