

Comparative response to nitrogen deficiency of a tropical and temperate grass in the interrelation between photosynthesis, growth, and the accumulation of non-structural carbohydrate

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Summary

A tropical grass, *Panicum maximum* var. *trichoglume* and a temperate grass, *Lolium perenne*, were grown in controlled environment rooms at near optimum temperatures of 25 and 18 °C, respectively. After an initial period of growth on full nitrogen nutrient solution, a proportion of the plants of both species were transferred to 'nil' N nutrient solution. A sequence of destructive harvests was taken as the plants became increasingly N deficient, and calculations were made of relative growth rate (R_W), relative leaf area growth rate (R_A) and net assimilation rate per unit leaf area (E_A). On a similarly treated group of plants, net photosynthetic rate (P_N) and dark respiration rate of attached leaves were measured.

Organic and nitrate N were estimated on the photosynthesis-tested leaves, and on comparable leaves from the growth analysis plants at each harvest. Percentage total non-structural carbohydrate (%TNC) was estimated for *P. maximum* only.

P. maximum maintained a higher R_W and P_N than *L. perenne* at comparable percent leaf N. With increasing N stress, E_A of both species declined to a similar relative extent, but R_A of *P. maximum* decreased much less than that of *L. perenne*. This difference in response to N between the species in the balance of assimilate input and consumption in new growth may in part explain why under N deficiency published studies indicate that %TNC reach higher levels in *L. perenne* and other temperate grasses than in tropical grasses such as *P. maximum*.

Introduction

Wilson & Haydock (1971) suggested that at low levels of nitrogen supply, tropical grasses as a group grew relatively better than did temperate grasses. More detailed study (Wilson & Sandland 1975) confirmed the ability of a tropical grass, *Panicum*

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maximum var. *trichoglume*, to maintain a higher relative growth rate than the subtropically adapted species, *Pennisetum clandestinum* at low tissue nitrogen concentrations. The purpose of the above studies was to elucidate some of the reasons for the generally low protein content of tropical compared with temperate pasture grasses (e.g. French, 1957). To extend these studies it was considered desirable to make a direct comparison between a tropical and temperate grass in their response to nitrogen. Some aspects of such a comparison are presented here.

Another interesting feature of the comparative physiology of tropical and temperate pasture grasses associated with their growth response to nitrogen is the effect of such treatment on the accumulation of total non-structural carbohydrates. Temperate grasses almost invariably accumulate substantial concentrations of non-structural carbohydrates under a declining plane of nitrogen nutrition (e.g. Alberda, 1965; Smith, 1973). This effect has been attributed to growth rate being reduced relatively more than photosynthetic rate (Blaser et al., 1966). On a similar argument, the generally small effect of plant nitrogen status on non-structural carbohydrate accumulation in tropical grasses (Balasko & Smith, 1971; Dovrat et al., 1972; Bessems, 1973; Ford & Williams, 1973; Lechtenberg et al., 1973; Wilson, 1973, 1975) may indicate a closer balance in the tropical species between the relative change in growth rate and photosynthetic rate with declining nitrogen supply. The present work examines this point.

A tropical grass, *Panicum maximum* var. *trichoglume*, and a temperate grass, *Lolium perenne*, are compared in their plant growth and leaf photosynthesis characteristics in relation to change in tissue nitrogen concentrations. The paper considers the implications of these responses to accumulation of non-structural carbohydrates.

Methods

General

These experiments were carried out in the controlled environment rooms of the IBS at Wageningen, The Netherlands, and details of cultural conditions, growth analysis, photosynthesis measurements and procedures for estimation of nitrogen and total non-structural carbohydrates are given in an earlier paper (Wilson, 1975).

Specific Detail

Plants of the tropical (C_4) grass *P. maximum* var. *trichoglume* cv. Petrie were grown singly from seedlings at 25 °C in one-litre pots on half-Hoagland's nutrient solution. An initial harvest was taken on day 28 from the sowing of seed. On day 30, the nutrient solution in one-half the number of pots was replaced by a 'nil' N nutrient solution (Wilson, 1975) for the remainder of the experiment; these plants were thus exposed to increasing N stress and became progressively more N deficient as the experiment progressed. The remainder of the plants continued on half-Hoagland's nutrient with frequent solution changes to ensure a high level of N supply. Harvests of whole plants from the plus (+N) and minus (—N) nitrogen

treatments (two replicate plants per treatment) were made on days 32, 34, 36, 38, 40 and 42 from sowing, i.e. after 2, 4, 6, 8, 10 and 12 days of treatment.

Plants of the temperate (C_3) grass *L. perenne*, vegetatively propagated by tillers from a clone of a tetraploid variety, were grown for about four weeks in half-Hoagland's nutrient solution at 18 °C with two plants per one-litre pot. When the plants were of comparable size to those of *P. maximum*, +N and —N treatments were commenced as described above, and harvests of whole plants were taken after 1, 4, 11, 15 and 18 days of treatment. Plant material from the two plants per pot was bulked at harvest and there were two replicate pots per treatment.

Measurements taken at harvest for both species were total leaf area per plant (excluding dead leaves), with an electronic planimeter, and whole plant dry weight after oven drying for 24 hours at 70 °C. Also, from each plant a separate sample comprising the last fully expanded leaf blade from several main tillers was taken for analysis of nitrate and organic N content; these leaves were equivalent in development stage to those used for photosynthesis measurements. The whole plant samples of *P. maximum* were analysed for total non-structural carbohydrates (TNC).

Net photosynthetic rate (P_N) measurements of attached leaves (two replicate chambers, 6 leaves per chamber) were made as described by Wilson (1975), with for both species an additional group of plants grown concurrently with the plants harvested for growth analysis. Light response curves for P_N were measured for leaves after plants had been exposed to varying numbers of days of nil N nutrient solution, viz 0, 3, 6, 9 and 12 days (*P. maximum*), and 0, 3, 6, 12 and 18 days (*L. perenne*); the test temperature was 25 and 18 °C, for the two species, respectively. After each photosynthesis test, the leaf material in the chamber was oven-dried and analysed for N content.

Treatment effects on the basic data are presented and then in Fig. 3 an integrated comparison is made in which growth and photosynthesis parameters for plants of varying degree of N deficiency are expressed relative to the comparable value for plants under full N nutrition. For this comparison, the P_N value for an irradiation level equivalent to that in the growth rooms ($70 \text{ J m}^{-2} \text{ s}^{-1}$) was determined by interpolation on the light response curves shown in Fig. 2. By interpolation on the growth curves, relative growth rate of whole plants (R_W), relative leaf area growth rate (R_A) and net assimilation rate per unit leaf area (E_A) were derived for each day on which P_N was measured, i.e. after an equivalent number of days on nil N solution. The values for the —N treatments were expressed as a percentage of the value for the +N treatment on the same day. The data were then plotted on the basis of leaf N content (%) of the —N plants.

Results

The +N plants grew vigorously throughout the experiment whereas those on the —N solution became progressively paler green, and many leaves were completely yellow after 12 and 18 days treatment in *P. maximum* and *L. perenne*, respectively. Compared with *L. perenne*, the % organic N in leaves of *P. maximum* was lower

RESPONSE TO NITROGEN DEFICIENCY OF TROPICAL AND TEMPERATE GRASS

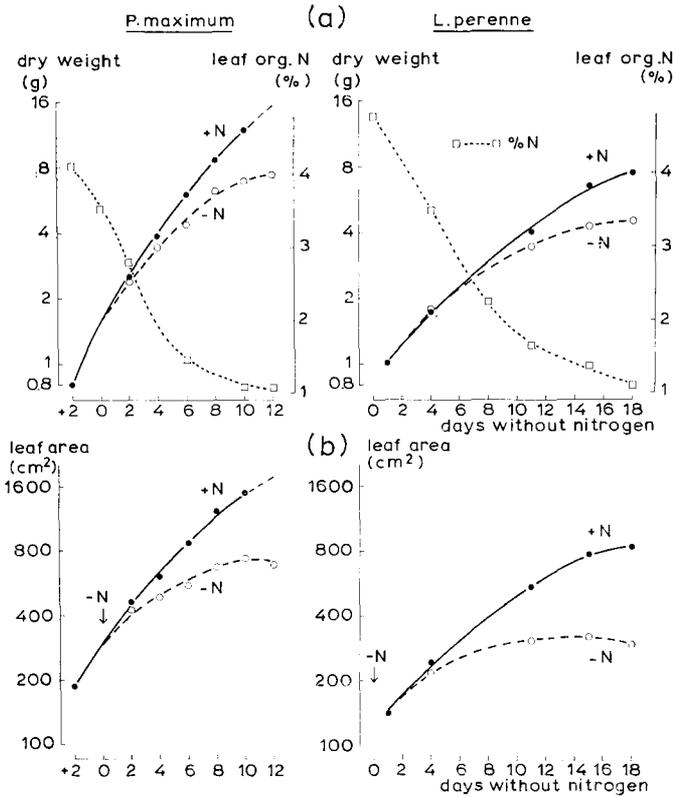


Fig. 1. (a) Whole plant dry weight and (b) total green leaf area per plant, of *P. maximum* and *L. perenne* under high (+N) and declining (—N) nitrogen supply plotted on a logarithm scale. The % organic N in leaves of the —N plants is also shown in (a).

for the +N plants and declined much more rapidly with time of exposure to —N solution (Fig. 1).

The +N plants of *P. maximum* had a higher relative rate of growth of dry weight and area than *L. perenne* (Fig. 1) and this is demonstrated by the average linear regression coefficients with time for log. dry weight *P. maximum*, 0.094 > *L. perenne*, 0.052 ($P < 0.001$) and log. leaf area *P. maximum*, 0.073 > *L. perenne* 0.046 ($P < 0.001$). This species difference was maintained in the —N treatment although growth rate in both species was markedly reduced ($P < 0.001$). Nitrogen stress had a relatively more severe effect on rate of leaf area increase than on dry weight increase.

Leaf P_N of +N *P. maximum* plants was much higher than that of *L. perenne* (Fig. 2). The P_N declined progressively in both species with time in —N solution. The rate of decline was much more rapid in *P. maximum* than in *L. perenne* and as a consequence the rates of *P. maximum* and *L. perenne* were similar, after 9 and

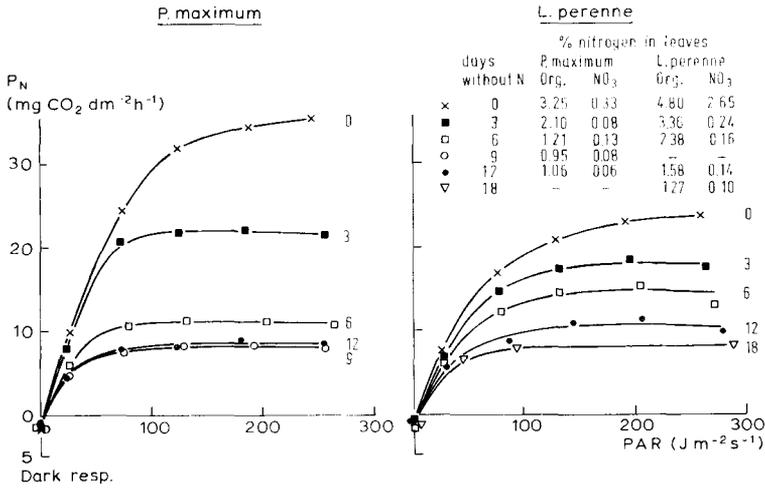


Fig. 2. Net photosynthesis (P_N) – photosynthetically active radiation (PAR) response curves of leaves on *P. maximum* and *L. perenne* plants grown for varying number of days on nil N nutrient solution. Inset shows organic and nitrate N content (%) of the leaves.

18 days without nitrogen, respectively. However, % organic N content declined to a low value much more rapidly in *P. maximum* than in *L. perenne*, and after 9 days without N the leaves of *P. maximum* were lower in % organic N than those of *L. perenne* after 18 days treatment (Fig. 2). The % $\text{NO}_3\text{-N}$ in the leaves quickly fell to low values in both species.

Fig. 3 shows the effect of increasing N stress on the growth and photosynthetic

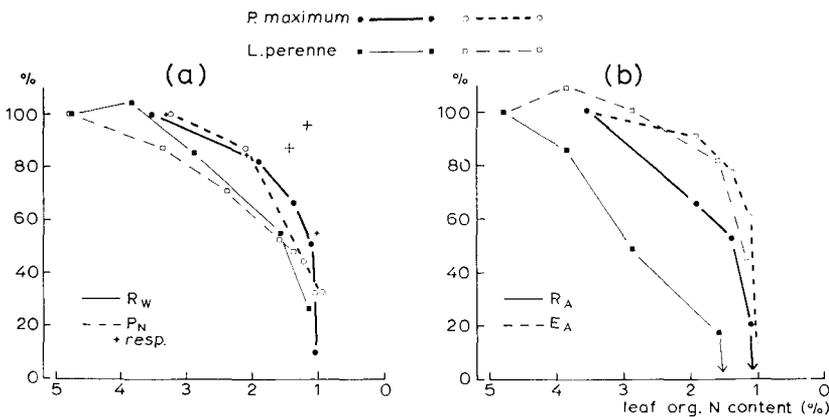


Fig. 3. Comparative effect for *P. maximum* and *L. perenne* of increasing N deficiency of the —N plants on (a) whole plant relative growth rate (R_W) and leaf net photosynthesis (P_N); (b) relative leaf area growth rate (R_A) and net assimilation rate per unit leaf area (E_A). Data expressed relative to the comparable value for the high N treatment.

parameters of the —N plants of each species, with the values expressed relative to the comparable values for the +N treatment. Within either species, R_W and leaf P_N were affected to a similar relative extent by progressive increase in nitrogen stress (Fig. 3a). The decline in these parameters with increasing nitrogen stress, however, was relatively less for *P. maximum* than for *L. perenne*, at least until the plants were severely N deficient at about 1.0% leaf organic N content. A more marked difference between the species is apparent when treatment effects on E_A and R_A are considered (Fig. 3b). Whilst the relative effect of nitrogen stress on E_A was comparable for both species, R_A was much more severely reduced in *L. perenne* than in *P. maximum* and hence the ratio between E_A and R_A was much more disturbed in the former species. At the lowest % leaf organic N, in both species there was an actual loss of green leaf area (Fig. 1), hence the R_A values were negative, and the relative values are not plotted in Fig. 3b.

Dark respiration rate of leaves in *P. maximum* tended to be less affected than P_N and other growth parameters by increasing nitrogen stress (Fig. 3a). Comparable data for *L. perenne* are not presented on a relative basis because they were variable. The values for *L. perenne* ranged between 0.5 and 1.2 mg CO₂ dm⁻² h⁻¹ over the various levels of N deficiency, with an average of 0.8 mg CO₂ dm⁻² h⁻¹. The average value for *P. maximum* was 1.3 mg CO₂ dm⁻² h⁻¹.

Nitrogen stress resulted in a small increase ($P < 0.01$) in the %TNC accumulated by whole plants of *P. maximum* (Table 1). The stimulation of carbohydrate accumulation appeared to decline with increasing severity of N deficiency.

Discussion

L. perenne was grown at a temperature which from experience was near optimum for this species. Similarly, *P. maximum* was also grown at a temperature near optimum for the present light conditions (Wilson, 1975), although with higher levels of irradiation more active photosynthesis and growth may be achieved at temperature above 25 °C (Ludlow & Wilson, 1971). Under these favourable temperature regimes and with good nitrogen nutrition, the R_W of *P. maximum* was considerably higher than that of *L. perenne*, the difference being associated with both higher leaf P_N , and R_A for *P. maximum*.

Table 1. Effect of nitrogen treatment on the accumulation of total non-structural carbohydrates (TNC) in *Panicum maximum*.

Period of treatment (days)	Nitrogen in —N leaves (%)	TNC		Relative value —N/+N (%)
		+N (%)	—N (%)	
2	2.80	7.3	11.2	153
6	1.46	10.5	17.3	165
10	1.07	9.4	11.4	121
12	1.05	9.0	10.3	114

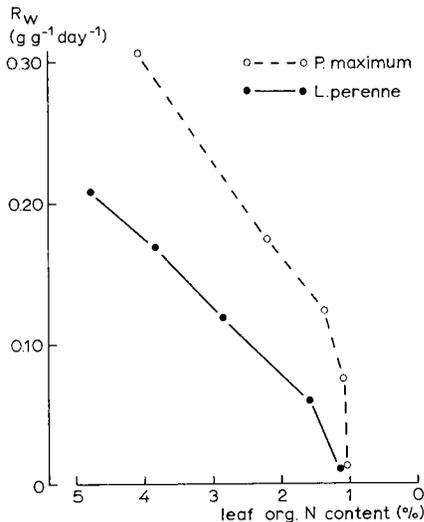


Fig. 4. Relation between plant relative growth rate (R_W) and leaf organic N content (%) for the —N plants of *P. maximum* and *L. perenne*.

As nitrogen stress was imposed the growth rate of both species declined, but the relative decline in R_W (Fig. 3) was less for *P. maximum* than for *L. perenne*. Although with severe N deficiency, as might be expected, the difference between species was reduced. The growth comparison between *P. maximum* and *L. perenne* when expressed as actual R_W plotted against per cent leaf organic N (Fig. 4) was similar to the comparison between *P. maximum* and *P. clandestinum* presented by Wilson & Sandland (1975). The present data thus extend the conclusions of Wilson and Sandland that the generally low protein content of tropical grasses results in part from their inherent physiological capacity to more efficiently utilize nitrogen. Coupled with this, the high potential R_W of tropical grasses leads to more rapid exhaustion of available nitrogen, as is clearly evident in this study in the rapid decline of per cent leaf organic N to very low values in the *P. maximum* plants used for the photosynthesis tests (Fig. 2).

The basis for the growth advantage of the —N plants of *P. maximum* over those of *L. perenne* appears to lie largely in the lesser effect of N deficiency on R_A in the former species. Leaf P_N in *P. maximum* was also less affected by increasing N stress than that of *L. perenne* but this advantage was offset by the apparently higher dark respiration rate of *P. maximum*. As a consequence, the net input of assimilates per unit leaf area per day, E_A , was affected by N stress to the same relative extent in both species (Fig. 3). The assimilates, however, were more effectively distributed to achieve continuing productive potential via leaf area expansion in *P. maximum*. This would agree with the general observation from a wider range of species, that over varying levels of applied N, a higher top/root ratio was maintained in tropical than in temperate grasses (Wilson & Haydock, 1971).

These species differences in growth response to increasing N deficiency have significant implications for the accumulation of carbohydrate reserves. Whilst the assimilatory capacity of both species, viz E_A , was affected to a similar relative extent by N deficiency, *P. maximum* continued to channel assimilates into new leaf area production much more effectively than did *L. perenne*. Consequently, the relative excess of assimilates above those consumed in growth was likely to be much lower for *P. maximum* and indeed the actual increase in TNC in this species with increasing N deficiency was small (Table 1). Levels of TNC were not measured for *L. perenne* because very detailed studies using the same variety, controlled environment facilities, and irradiation level, were made by Alberda (1965). These studies revealed accumulation of TNC up to 25–35 % of dry weight in leaves from plants grown at 15–20 °C under increasing N deficiency and at tissue $\text{NO}_3\text{—N}$ percentages comparable to the lower values for *L. perenne* in the present work. Further research on *L. perenne* and other temperate grasses, reviewed by Smith (1973), support these findings.

There is little evidence to suggest that the use of other growth temperatures would obviate the difference between *P. maximum* and *L. perenne* in TNC accumulation under N deficiency. A previous study (Wilson, 1975) indicates that the level of TNC in low N plants of *P. maximum* is relatively insensitive to change in temperature. On the other hand, the data of Alberda (1965) do indicate that for *L. perenne* the %TNC accumulated under N deficiency decreases with higher growth temperature. At the temperature at which *P. maximum* was grown in this experiment, viz 25 °C, the TNC in leaves of *L. perenne* was up to 17–24 % of dry weight, and in stubble up to 30 %. These values are still higher than the 10–11 % TNC in the severely deficient plants of *P. maximum* (Table 1). At temperatures below the 18 °C used here for *L. perenne*, TNC increased up to 40 % of dry weight in the plants of Alberda (1965).

An additional factor influencing the accumulation of TNC in *P. maximum* associated with high R_W , was the more rapid decline in N status of this species compared with *L. perenne*. A critical state of N deficiency was reached in 9 days in *P. maximum* but took 18 days in *L. perenne*. There is some evidence from Table 1 and from previous work (Wilson, 1973) that in *P. maximum* some TNC tends to accumulate under moderate N deficiency but not when N deficiency is severe. This might perhaps be expected because at values less than 1.4 % organic N for the most recently expanded leaf of *P. maximum*, the rate of assimilate input (E_A) declined abruptly. This may be not so evident from P_N but this measurement was made on the youngest expanded leaf and older leaves would be significantly more deficient in nitrogen. The growth parameters for *L. perenne* showed trends similar to those for *P. maximum* but because N deficiency developed more slowly, this species had a longer period in which to accumulate reserve carbohydrate.

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