RESEARCH ARTICLE

How much do water deficits alter the nitrogen nutrition status of forage crops?

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Abstract Water deficits alter the nitrogen nutrition of crops. In grasslands, this has a major impact on both forage yield and nitrogen fluxes in the soil. It is important to assess the N balance in order to adjust fertilization to the expected needs of the crop and thus minimize any environmentally negative impacts of crops. Grassland species, including grasses, display a diverse ability to utilise soil resources. Nitrogen fluxes and the nitrogen absorption by grass swards of two species with contrasting rooting depths were computed using the appropriate module from the STICS simulation platform. In the case of the deep-rooted species, tall fescue, soil mineral N fluxes to the roots were very close to N uptake values, consistent with its nitrogen nutrition index being lower than one. In the case of the shallow-rooted species Italian ryegrass, there was a large excess in terms of N supply, which was also consistent with its non-limiting nitrogen nutrition index. In both species, and even when nitrogen demands for growth were fully satisfied, the nitrogen nutrition index was closely and linearly related to the soil mineral N flux to roots.

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Introduction

Most grasslands, which cover a very large proportion of continental land areas, are subject to regular and sometimes severe water deficits. Drought can also reduce the use of soil mineral nitrogen, which results in poorer forage quality (Onillon et al. 1995). Increasing grassland productivity while protecting the environment requires the management of both water and mineral fertilisation based on accurate data (Rodriguez et al. 2009). In order to better understand the quantitative impact of water deficits on the nitrogen cycle at the field level is therefore a prerequisite to the cropping of sustainable intensive grasslands.

Water deficit, nitrogen deficit, or a combination of both, may limit the productivity of a crop throughout its life cycle (Sadras 2005). In grasslands, as in all crops, the effects of water and/or nitrogen deficit on plant production are therefore closely dependent on their timing and intensity (Bradford and Hsiao 1982; Nielsen and Halvorson 1991). As the major solvent and transport agent at all organizational levels of the agro-ecosystem, water controls the entire biogeo-chemical cycle of nitrogen and ultimately its

availability for plant production (e.g.: Smika et al. 1965; Lemaire and Denoix 1987; Akmal and Janssens 2004).

Firstly, because of its direct impacts of water deficits on plant growth, the amount of nitrogen absorbed by crops is reduced under dry conditions, even when reduced nitrogen needs of plants are fully satisfied. Secondly, under dry soil conditions, soil nitrogen may become unavailable in the rhizosphere so that yield may be further reduced (Gonzalez-Dugo et al. 2005). These direct and indirect limitations of water shortage on plant growth render complex any quantification of the effect of water deficit on nitrogen nutrition.

A previous study on wheat by Devienne-Baret et al. (2000) related the nitrogen uptake of wheat (*Triticum aestivum*) to the soil mineral *concentration*. However, this relationship was shown to be unstable, dependent as it is on a variety of environmental and management factors (Farruggia et al. 2004). Among the essential variables involved, water fluxes in soil clearly exert a major impact on nitrogen availability, as suggested by previous experiments (Garwood and Williams 1967; D'Aoust and Tayler 1968). It therefore seems appropriate to consider the mineral nitrogen *flux* to the roots. Gonzalez-Dugo et al. (2005) analysed the impact of water deficit on the nitrogen status of *Festuca arundinacea* (*Fa*) and *Lolium multiflorum* (*Lm*). They showed a clear difference between the two species in terms of both the degree of impact of drought on the nitrogen economy of the crop itself, and its ability to recover from a dry period. Specific differences were ascribed firstly, to differences in the crop growth rate at the time of the experiment and secondly to the difference in rooting depth, *Lm* having a much shallower root system than *Fa*. However, that study did not provide any quantitative analysis of mineral nitrogen fluxes and availability for growth because it was limited to 1 year and a single sequence of drought and recovery, so that intermediate measurements during the regrowth were not performed. The experiment was therefore pursued for a second year in order to collect more data on soil humidity, mineral nitrogen concentrations and plant composition, which are thus presented in this paper.

In order to compute mineral N fluxes to roots from the experimental data, equations from the appropriate routine of the generic crop simulation model, STICS (Brisson et al. 1998, 2009) can be used. The routine considers the contribution of a number of soil layers to transpiration fluxes on the one hand and local mineral nitrogen concentrations in the same soil layer (Brisson et al. 2009) on the other. These fluxes include convection and diffusion because the latter may contribute to the total mineral N flux under drier conditions (Raynaud and Leadley 2004). To our knowledge, an estimate of the actual nitrogen flux to the roots has never been compared with N uptake based on a total dry matter balance.

However, neither the N flux nor N uptake provide any quantification of nitrogen *deficiency* at the crop level. The nitrogen status of a crop is best described by the ratio between the actual herbage nitrogen concentration (g N g^{-1} above-ground biomass) and a critical concentration. Various attempts to define this critical concentration have been published in the past (e.g. Van Dobben 1962). In a recent synthesis, Lemaire and Gastal (2009) used above-ground biomass to define the critical nitrogen concentration (see also: Colnenne et al. 1998; Hermann and Taube 2004; Bélanger et al. 2001; Ziadi et al. 2008). The critical nitrogen concentration can be defined as the minimum N concentration of green above-ground biomass that enables the maximum primary production of the crop (Greenwood et al. 1986). Using that approach, the critical N concentration is mainly dependent on standing biomass, according to a negative power function, that is sometimes called a dilution curve (Lemaire and Salette 1984; Lemaire and Gastal 2009). Knowing the soil N flux to roots and uptake on the one hand, and the actual N_{NI} of the crop on the other hand, makes it possible to determine the extent to which the soil mineral N contribution limits growth under water stress.

The aim of the work reported here was thus (1) to establish the nitrogen balance of grass swards of two contrasted species with different root systems, growing on the same soil, (2) to compare this balance to the theoretical mineral nitrogen flux to roots, and (3) to interpret the resulting variations in nitrogen status observed during periods of controlled water deficit and recovery in the field.

Materials and methods

Experiment

The experimental design was described in Gonzalez-Dugo et al. (2005). The experiment was run during

the summers of 2003 and 2004 with the same treatments and conditions. Briefly, 24 plots of *Festuca arundinacea* (*Fa*, *cv.* Florine) and *Lolium multiflorum* (*Lm*, *cv.* Tonyl), each covering 18.75 m² (5 m × 3.75 m), were sown by hand on September 11, 2002. Under the dry treatments, *Lm* swards were too degraded after the autumn of 2003 to be considered for further study in 2004. Until June 16, 2003, all plots were managed the same way, receiving low N fertilisation of 40 kg N ha⁻¹ at each of the first three cuts. Following the cut in June, four contrasted treatments were applied: two irrigated (N+/Irr and N−/Irr), and two dry (N+/Dry and N−/Dry). Each treatment was repeated three times. On June 16, 2003 and June 14, 2004, the plots were cut. Half of the plots received 160 kg ha⁻¹ mineral-N, while the other half received 40 kg ha⁻¹ mineral-N only. One half was irrigated afterwards to meet potential evapotranspiration needs (ET$_0$, computed according to the Penman–Monteith formula for reference evapotranspiration), while the other half was protected by rainout shelters. The water treatments were split into two blocks. During both years, the shelters remained open for 90% of time and were only placed over the swards if it was raining. On June 30, 2003 and July 7, 2004, following a period of drought, the dry plots remained uncovered until July 15 (both years), and thus received 54 and 58 mm in 2003 and 2004, respectively. Between the two summer experiments, two applications of nitrogen fertilizer were made, also of 40 kg Ha⁻¹ N each time. Three cuts to 7 cm stubble height were performed during the same period.

Soil measurements

The soil, a cambisol (WRB 2006) referred to as *terres rouges à chataigners* (Ducloux and Chesseron 1989; Hubert 2009), lies on an ancient ferrallitic soil. The brown surface horizon is approximately 1 m deep with a flint bank at a depth of approximately 0.8 m. The silt content increases from top to bottom from 23 to 47% with a homogeneous sand content of 20% within the soil profile, which contains iron nodules (<3 mm in diameter) that are increasingly abundant from top to bottom. The carbon content of the top horizon is approximately 8 g kg⁻¹ in the top 30 cm and 2 g kg⁻¹ at a depth of 1 m.

Access tubes for a neutron probe (CPN 503) were inserted in all dry plots (12 tubes) to a depth of 1 m, thus enabling repeated measurements of volumetric humidity. On June 24 and July 8, 2003, and June 16 and July 1, 8, 15 and 26, 2004, soil samples of approximately 250 g fresh weight per plot (each mixed sample consisting of three cores per plot) were collected from all plots at four depths (0–25, 25–50, 50–75 and 75–90 cm) for the analysis of mineral nitrogen (NO$_3$-N and NH$_4$-N).

Plant material

During the two summer periods, predawn leaf water potential (Ψ_1) values were measured weekly on the last fully expanded leaf using a pressure chamber (Scholander et al. 1965) in three tillers per plot and per species. On June 25 and July 9, 2003, and June 16 and July 1, 8, 15 and 22, 2004, two samples of 380 cm² of sward were collected from each plot and included the total above-ground biomass and soil to a depth of a few centimetres. Following separation, the lamina, sheath and litter dry weight were measured after 48 h in a ventilated oven at 60°C. On June 24 and July 8, 2003, and June 16 and July 22, 2004, 8-cm diameter soil cores (one per plot in 2003 and two per plot in 2004) were extracted from each plot at depths of 0–25, 25–50, 50–75 and 75–100 cm, and stored in the freezer (−18°C) until analysis. These soil cores were soaked overnight in 10 l of salted water (∼55 g l⁻¹ NaCl) and the roots were washed over a 1-mm mesh filter prior to thorough hand separation. The roots were then oven-dried at 60°C for 48 h, before being ground so that they could pass through a 1-mm mesh. The C an N concentrations (g N g dry matter⁻¹) of the root samples were measured with a C–N elemental analyser (model NA 2000; Carlo Erba, Milan, Italy). The nitrogen content of the root compartment in each soil layer (g m⁻²) was then computed as the product of the total root dry matter weight by its nitrogen concentration and divided by the surface area of the core (5.03 10⁻³ m²).

To enable repeated measurements of the nitrogen nutrition index (N_{NI}) in the plots without destroying too much material, two handfuls of leaves were sampled randomly from each plot and cut with scissors at 10 cm from the tip of the longest leaf; dead material was discarded and the laminae were

oven-dried for 48 h at 80°C, following the procedure detailed in Farruggia et al. 2004. Leaf samples were ground in the same way as the roots and their nitrogen concentration was analysed using the C–N elemental analyser. The nitrogen nutrition index was then computed as in Farruggia et al. 2004 and also in Duru (2004), as follows:

$$N_{\mathrm{NI}} = \frac{N_{\mathrm{sup}}}{3} - 0.32 \tag{1}$$

where N_{sup} is the nitrogen concentration (mg N g leaf dry matter^{-1}) of the lamina exposed to direct incident radiation in the canopy.

Mineral nitrogen flux in the soil

Computing of the soil nitrogen flux to grass roots was performed using the equations of the STICS crop simulation model (Brisson et al. 2009). Both convection and diffusion fluxes were taken into account. The convective flow was equal to the product of the soil solution nitrogen concentration times the transpiration flux in each soil layer. For plots under water deficit, the hypothesis was made that vertical movements were negligible over the periods of time considered. Given that the water table was much lower in the subsoil (typically more than 20 m below the root front in the field plot), it was assumed that capillary rise also was negligible. Hence, for each soil layer, the convective flow was derived from changes in soil volumetric humidity measured with the neutron probe and the total mineral N concentration, thus:

$$F_{Cj} = \sum_i \frac{N_{ij}}{H_{ij}} \times \frac{(H_{ij} - H_{(i-1)j})}{n} \tag{2}$$

where F_{Cj} was the convection flux in the horizon j (mg m^{-2} day^{-1}), N_{ij} the soil nitrogen content of soil layer j on day i (mg m^{-2}), H$_i$ was the volumetric soil humidity and n was the number of days between two measurements of H_i and N_i.

Under irrigated treatments, the transpiration flux was partitioned in the soil depending on the root length density measured at each soil layer (Maertens et al. 1974; Sharp and Davies 1985).

At each soil depth, the diffusion flux was computed using the Brisson et al. (2009) equation, which stems from the approach described by Gardner (1960), together with a simplified version of the

relationships given in Barber and Peterson (1995). In each soil layer, the variables controlling the diffusion flux (mg m^{-2} day^{-1}) were (1) the difference in the total mineral N (NH$_4^+$ and NO$_3^-$) concentration in the soil solution between the soil and the root surface, (2) the root surface area, (3) the distance between roots, (4) the diffusivity of mineral nitrogen solutes in soil water channels, and (5) soil humidity. As in Brisson et al. (2009), it was assumed that roots were vertical and equidistant, and that the mineral N concentration (equal to the measured concentration) decreased linearly from the middle of two adjacent roots to the surface of the roots where it was supposed to be nil. Diffusion was only supposed to vary with soil humidity:

$$F_{Dj} = \frac{\sum_i 4\sqrt{\pi} \times D \times r_{ij} \times \sqrt{d_{ij}} \times [N]_{ij} \times 100}{n} \tag{3}$$

where F_{Dj} was the mean daily diffusion flux at horizon j (mg m^{-2} day^{-1}), D was the maximum nitrate diffusion coefficient in soil water films (0.018 cm^2 day^{-1}, Brisson et al. 2009), r_{ij} was the proportion of available soil water, d_{ij} was the root length density in horizon j on day i (cm cm^{-3}), $[N]_{ij}$ was the mineral N concentration of the soil solution (kg ha^{-1} mm^{-1}), and n was the number of days between two measurements. Daily values of $[N]_{ij}$ were interpolated between two successive measurements. r_{ij} was computed as:

$$r_{ij} = \frac{(H_{ij} - P_{\mathrm{FP}j})}{(C_{Cj} - P_{\mathrm{FP}j})} \tag{4}$$

where $P_{\mathrm{FP}j}$ and C_{Cj} were the humidity at the permanent wilting point and field capacity, respectively, in horizon j. Daily values of H_{ij} where interpolated between two successive measurements. $P_{\mathrm{FP}j}$ and C_{Cj} were derived from the texture and soil carbon concentration of each soil layer (Campbell 1974).

Hence, the total daily diffusion and convection fluxes, respectively F_D and F_C (mg m^{-2} day^{-1}), were summed over all soil layers as follows:

$$F_D = \sum_j F_{Dj} \tag{5}$$

and

$$F_C = \sum_j F_{Cj}. \tag{6}$$

Results

Climate

During the 2004 experiment, the climate was cooler and wetter than in 2003 and solar irradiance was also somewhat lower, resulting in lower ET_0 values (Fig. 1). This was particularly the case during the recovery period during 2004, when the daily mean air temperature, irradiance and ET_0 were 16°C, 1,962 J cm^{-2} and 3.9 mm, respectively, rather than 18°C, 2,490 J cm^{-2} and 5.2 mm during the previous period of water deficits. During this recovery period of the 2004 experiment, the climatic conditions were also colder and cloudier, i.e. less favourable to sward growth than in 2003 during the same phase of the experiment.
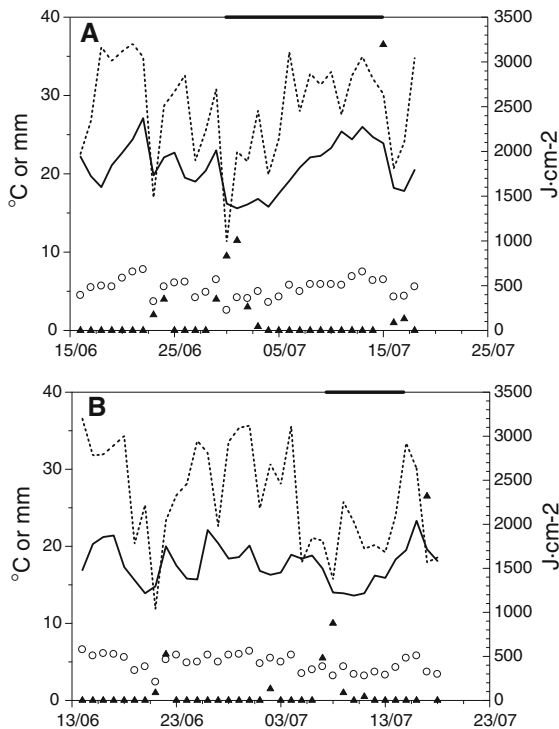
Fig. 1 Rainfall (mm): *closed triangles*, mean air temperature (°C): *solid line*, ET_0 (mm): *opened circles* and Global Irradiance (J cm^{-2}): *dotted line*, for the periods studied in 2003 (**a**) and 2004 (**b**)

Water deficits

Plant water status

In 2004, as in 2003 (Gonzalez-Dugo et al. 2005), the mean predawn leaf water potential declined in tall fescue (Fa) during the two dry periods separated by a 7-day recovery period. The maximum values measured under irrigated treatments were approximately −0.2 MPa, which probably indicated some weak night transpiration (Fig. 2). In both years and both species, nitrogen fertilisation treatments induced no significant difference between ψ_d values. In 2004, minimum values reached −0.8 MPa at the end of the second dry period, i.e. slightly higher than in 2003. Measurements of soil humidity under the dry treatment were used to compute evapotranspiration (ET). This was higher in 2004 during the period studied than in 2003, but the difference between the reference evapotranspiration value (ET_0) and ET was greater in 2003 (Fig. 3). At the end of the second dry period of that year, ET was only 20% of ET_0. This was consistent with the lowest value of predawn leaf water potentials observed during that period (Fig. 2). In general, during the 2003 summer, ET was close to or slightly lower in Lm than in Fa, again, which was consistent with the slightly higher ψ_d value measured in that species (Gonzalez-Dugo et al. 2005).

Above-ground crop growth

Sward dry matter was measured twice in 2003 (Gonzalez-Dugo et al. 2005) and five times during regrowth in 2004 (Fig. 4a). The first dry period induced a significant decline in growth rates with both nitrogen fertilisation rates. Following the application of irrigation between July 7 and July 15, only the low N dry treatment displayed a higher growth rate. Indeed, previously irrigated treatments tended to show a reduction in growth rate because of low levels of temperature and radiation. This resulted in very similar quantities of above-ground biomass of the swards at the end of the 2004 experiment, with only the N+/Irr treatment displaying significantly higher values.

The leaf area index (Fig. 4b) was followed in 2004. The effect of the dry treatment was very marked after July 2. N treatments also caused significant differences in the irrigated swards. In the
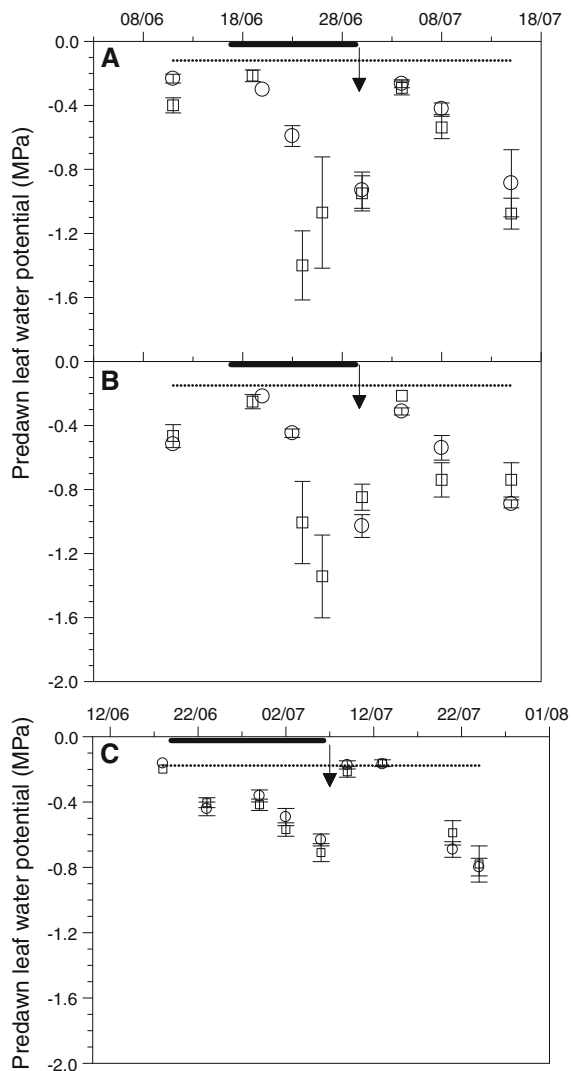
Fig. 2 Predawn leaf water potential (MPa) in 2003 [tall fescue (a) and Italian ryegrass (b)] and in 2004 [tall fescue (c)] for dry treatments under two N fertilization levels: N+ (*circles*) and N− (*squares*). Rainout shelters protected plots with dry treatments from the start of the experiments until rewatering periods (thick continuous line over *x*-axis). *Arrows* indicate 20 mm irrigation at the beginning of rewatering (measurement taken before irrigation). Mean values for irrigated treatments are shown as the *horizontal doted line* in each figure. *Bars* indicate ±standard error (n = 6)

dry plots and under low N fertilisation, the LAI value did not exceed 3. Following irrigation, the LAI recovered strongly under the N+/Dry treatment, reaching approximately the value of 4, while swards under the N−/Dry treatment only displayed a slight increase. Following the onset of the second dry period on July 15, any increase in the LAI was halted,

while continuously irrigated swards with a high N fertilization rate (N+/Irr) sustained high growth rates.

Root biomass and density

The root biomass was sampled twice in 2003 (Gonzalez-Dugo et al. 2005) and five times in 2004. The average carbon concentration (g C g root dry matter^{-1}) was $37 \pm 0.3\%$ for all root samples from both years. The carbon concentration of root material being of approximately 42% (Peri and Lasagno 2009), that was suggestive of slight contamination by soil mineral elements. In 2004, the root biomass of tall fescue had increased markedly when compared with the biomass measured during the summer of the previous year (Fig. 5a), but remained very stable during the summer period studied. Biomass was largely concentrated in the top 25 cm layer of the soil i.e. $\sim 70\%$ on average under all treatments (Fig. 5a). In contrast to 2003, fertilization treatment in 2004 induced significant differences in the distribution of roots, 66% of them being found in the top 25 cm soil layer under high N and dry treatments and around 75% under low N and irrigated treatments (data not shown). Taking the specific root length values found by Gonzalez-Dugo et al. (2005) for these plots, root length density was computed at different depths in each plot (Fig. 5b). Given the much finer roots of Italian ryegrass, both species had similar RLD at depth. In 2004, the RLD of tall fescue had nearly doubled by comparison with 2003, while non irrigated swards of *Lm* did not survive the summer of 2003.

Nitrogen content and uptake

Nitrogen levels in total biomass were highest in tall fescue (Figs. 6, 7), as was the quantity of biomass itself. The N fertilization rate had a greater impact than the water regime on the N content of swards. At the end of the experiments in both years, the high N irrigated treatment enabled an N content of approximately 15 g m^{-2}. In 2003, it was only under high N fertilisation that the difference in N content between the two water regimes was significant. The impact of water deficit was not significant in 2004 (Fig. 7). Lamina displayed the highest N content, while sheaths had the same responses to treatments as the lamina. Consistent with the increase in the root biomass of tall fescue plots
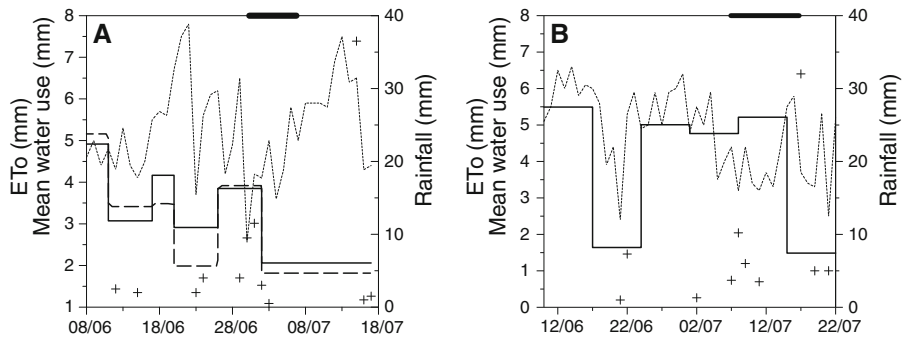
Fig. 3 Variations in daily reference evapotranspiration (ET_0, *dotted line*), rainfall (+, mm), and mean daily water use (mm) values for tall fescue (*continuous line*) and Italian ryegrass (*discontinuous line*) in 2003 (**a**) and 2004 (**b**) in swards subjected to water shortage in the field. Note that only tall fescue was considered in 2004
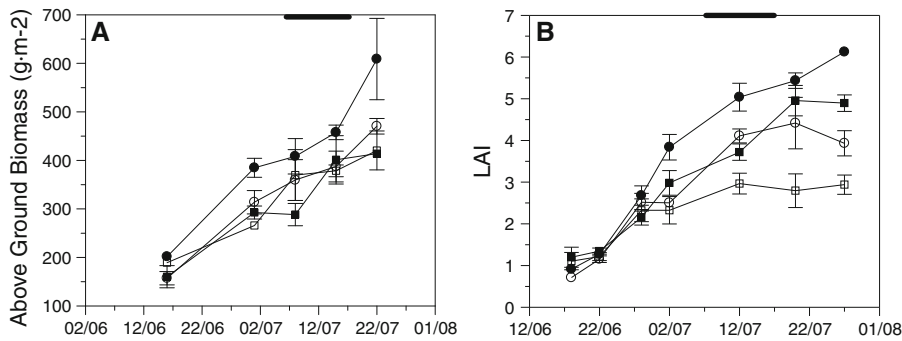

Fig. 4 Time course of **a** aboveground biomass (g m^{-2}) and **b** the leaf area index (*LAI*) of tall fescue plots in 2004 under two N fertilization levels: N+ (*circles*) and N− (*squares*), and two watering regimes: irrigated (*solid symbols*) and water shortage (*open symbols*). *Thick continuous lines* over the *x*-axis show the period when water-stressed swards were irrigated. *Bars* represent ±standard error ($n = 3$)
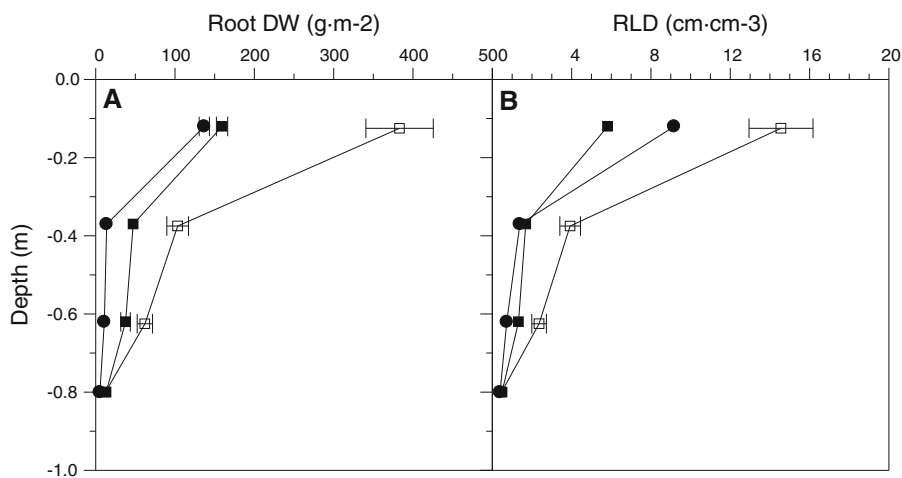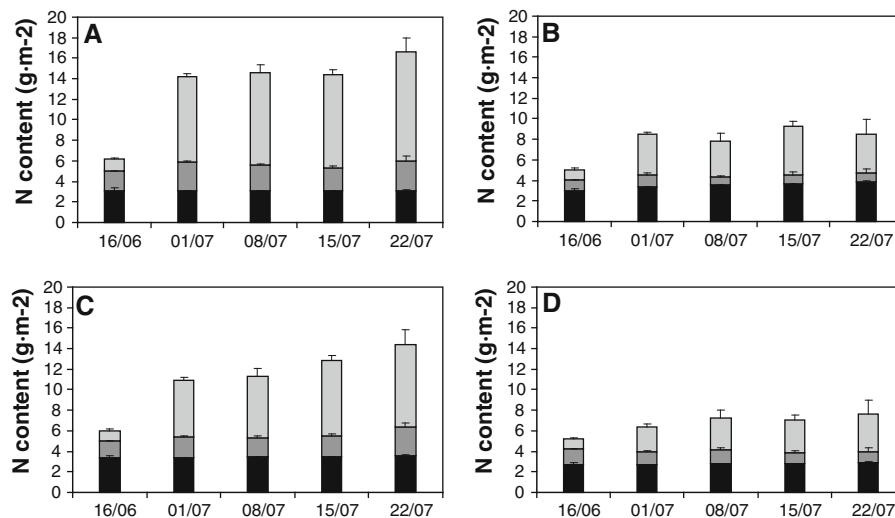

Fig. 5 **a** Mean value (averaged for the four treatments) of root biomass distribution (g m^{-2}) within the profile for tall fescue (*squares*) and Italian ryegrass (*circles*) swards. *Bars* indicate the ±standard error ($n = 3$). **b** Distribution of root length density (*RLD*, cm cm^{-3}) in the profile. *Closed symbols* 2003, *opened symbols* 2004
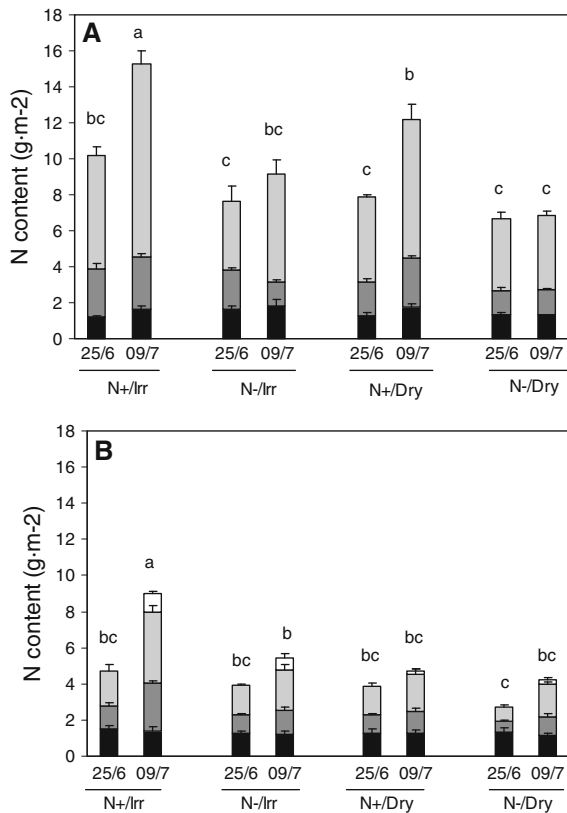
Fig. 6 N content (g m^{-2}) in different plant parts in swards of tall fescue (**a**) and Italian ryegrass (**b**) at two dates in 2003 under all treatments. *Bars* represent ±standard error (*n* = 3). For each species, total *N* values with the same letter are not statistically different (*P* > 0.05)
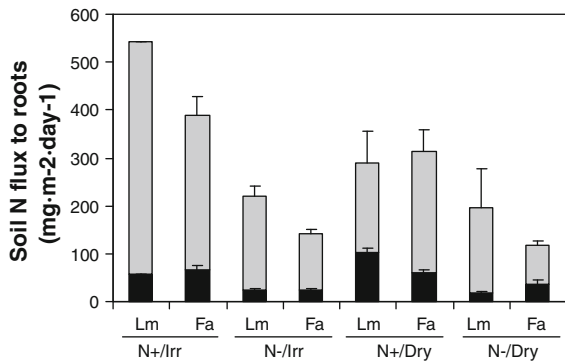
between 2003 and 2004, there was also a marked rise in root N levels in that species. However, in neither species did the root N content vary significantly between the beginning and end of regrowth studied during the summer.

Soil mineral nitrogen fluxes

Because of differences in soil mineral N concentrations (data not shown) and water consumption, the mineral nitrogen ($NO_3 + NH_4$) fluxes computed from the transpiration-induced convective flux and diffusive fluxes (Eqs. 2–6) differed markedly between treatments and species (Fig. 8). Fluxes ranged from about 550 mg^{-2} day^{-1} under N+ treatments to about 110 mg m^{-2} day^{-1} under low N fertilization rates. With each treatment, the differences in soil mineral N fluxes between species were not significant. In all cases, convection was the principal transport process (Fig. 8), with diffusion contributing an average of about 15%. However, the greatest contribution of diffusion, found with N+/Dry treatments, was ∼18 and 37% of the total flux in tall fescue and Italian ryegrass, respectively.

Using the changes to total plant N content observed when the fluxes were estimated, the total N uptake (N_a) was also estimated, under the assumption that no significant senescence occurred between two measurements. The results for the three periods

Fig. 7 N content (g m^{-2}) in different plant parts in swards of tall fescue at five dates in 2004 for N+/Irr (**a**), N−/Irr (**b**), N+/Dry (**c**) and N−/Dry (**d**). *Bars* represent ±standard error (*n* = 3)

Fig. 8 Soil mineral N flux to roots (mg m^{-2} day^{-1}) for Italian ryegrass (Lm) and tall fescue (Fa) separated by its two components: diffusive and convective. For tall fescue, the mean value for the three periods considered is represented
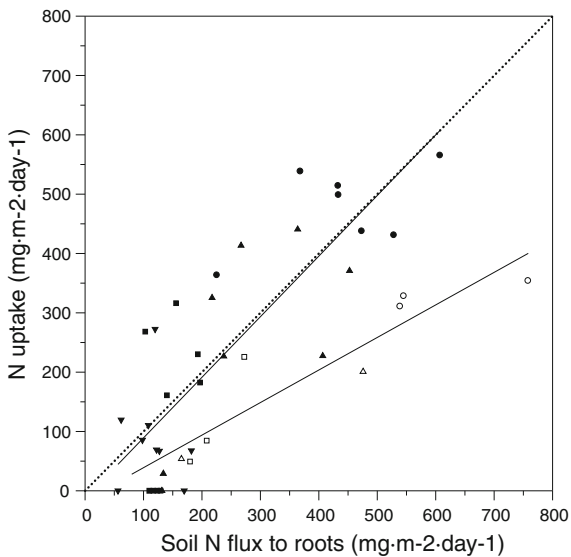
Fig. 9 Relationship between N exported by the crop (mg m^{-2} day^{-1}) and soil mineral N flux to the roots (mg m^{-2} day^{-1}) for tall fescue (*closed symbols*) and Italian ryegrass (*opened symbols*). Each *point* represents a single measurement from one plot during one of the considered periods. Linear regressions for tall fescue ($y = 1.021x - 13.05$; $R^2 = 0.67$; *continuous line*) and Italian ryegrass ($y = 0.548x - 15.88$; $R^2 = 0.87$; *discontinuous line*) are represented. The *dashed line* corresponds to the 1:1 line

considered for tall fescue, as well as the single period for Italian ryegrass, were then plotted versus the total mineral N fluxes to the roots of plants (Fig. 9). The linear regression equation for all periods and plots with tall fescue was:

$$N_a = 1.02(F_C + F_D) - 13 \tag{7}$$

$$R^2 = 0.674$$

where N_a was N uptake (mg m^{-2} day^{-1}). The slope of the regression did not differ statistically from 1, indicating that tall fescue probably absorbed and used most of the nitrogen transported through the soil to the roots.

In Italian ryegrass, the computed nitrogen flux was always much higher than the crop nitrogen balance, suggesting a considerable excess in the mineral N available in the rhizosphere:

$$N_a = 0.55(F_C + F_D) - 16 \tag{8}$$

$$R^2 = 0.8753$$

Italian ryegrass thus appeared to use only slightly more than half of the mineral N flux that was driven to the roots, irrespective of the treatments applied. However, the total mineral N flux to roots was even more closely and linearly related to the N absorbed by the plant during the period studied than in tall fescue, as indicated by the higher determination coefficient.

Crop nitrogen status

Crop nitrogen status was determined using the nitrogen nutrition index (Fig. 10). The results for 2003 were described in Gonzalez-Dugo et al. (2005) and highlighted the significant impact of water deficit on N_{NI} values (Fig. 10a). Such an impact was not observed in 2004 (Fig. 10b). But as in 2003, a marked effect of the N fertilization rate could be demonstrated. Once again, in 2004, the N_{NI} of *Fa* was lower than that of *Lm*, when N_{NI} always remained close to, or higher than 1. The lowest values were found in *Fa* at the end of regrowth in 2004, where the value was also lower at the beginning of the period studied than in 2003.

The relationships between the soil mineral N Flux to roots and the N_{NI} of plots at the end of each period considered were studied for each species. In both cases, the relationships were relatively tight and linear.

In Italian ryegrass, only one period could be studied with three replicates under each treatment (Fig. 11a). All points could be plotted along a single line, for which the direction coefficient differed significantly from 0. This suggested a prominent role
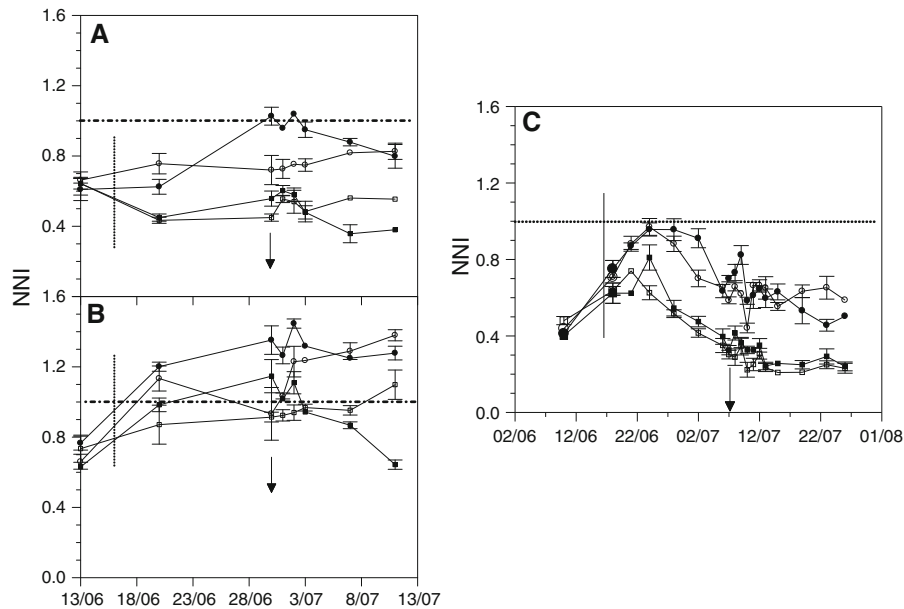
Fig. 10 Time course of the nitrogen nutrition index (N_{NI}) values during experiments under two N fertilization levels: N+ (*circles*) and N− (*squares*) of irrigated (*closed symbols*) and water shortage (*opened symbols*) for tall fescue (**a**) and Italian ryegrass (**b**) during 2003, and tall fescue in 2004 (**c**). *Bars* represent ±standard error ($n = 3$). The *arrows* indicate the day of rewatering and the *vertical dotted lines* the days of cutting and fertilization

of N flux in variations of N_{NI} values. It also indicated that despite slow growth and subsequent nitrogen demands for growth that were fully satisfied, N_{NI} still increased linearly beyond the optimal value of 1 with the mineral N supply to the roots.

In tall fescue, the relationship (Fig. 11b) was seen to be strikingly tight regarding data obtained under the N+/Irr treatment within a broad range of N_{NI} values ($R^2 = 0.89$). Under dry conditions, the correlation between N_{NI} and the N flux was weaker in that species, but the relationship was similar. Under low N fertilisation, the range of values for the soil N flux and N_{NI} was obviously smaller but the data could also be grouped along the same line as that for N+/Irr data.

Discussion

The 2 years studied differed in terms of their climate which thus induced differences between the responses of plants to the treatments. Drought was more pronounced in 2003, causing slower growth rates and greater differences between treatments. The recovery period induced by irrigation triggered similar and immediate responses of the crop water

status. Growth rates in 2004 also responded, but for a very short period, which was indeed followed by a complete absence of LAI growth.

N movements in the soil resulted mostly from the transpiration stream. Several variables were measured to compute the water flux and associated mineral N flux. Root length density was measured with less accuracy because of major uncertainty concerning the root biomass itself (Amato and Pardo 1994; Pierret et al. 2005) and specific root length (Gonzalez-Dugo et al. 2005). The root biomass almost doubled between 2003 and 2004, which was consistent with the slow rate of soil colonization by tall fescue. Durand and Ghesquière (2002) had also reported that root biomass increased over 2 years in tall fescue grown under similar conditions. Similar high values of root length densities are typical for grass (Gregory 2006). Despite differences in RLD and depth of water extraction between *Lm* and *Fa*, the leaf water potential of tall fescue and Italian ryegrass did not markedly differ, in contrast to what was found by Durand et al. (1997). This was probably due to the slow growth and low subsequent water demand of Italian ryegrass in 2003 (Gonzalez-Dugo et al. 2005).
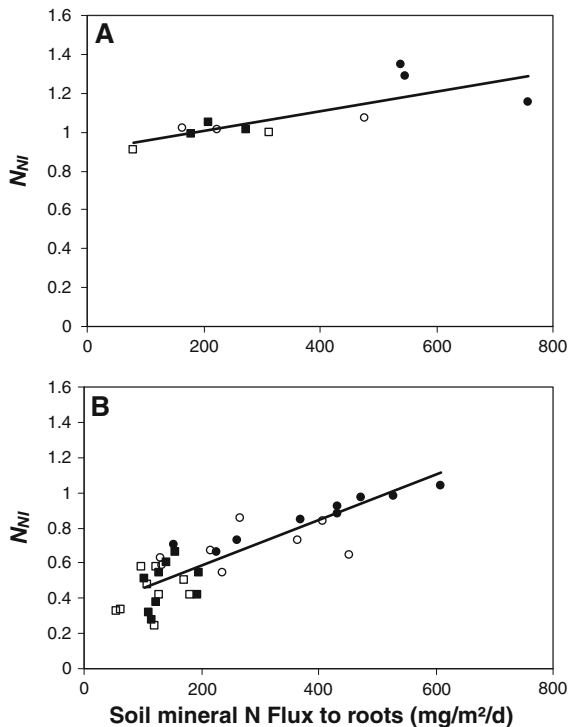
Fig. 11 Relationship between N_{NI} and soil mineral N flux to the roots (mg m^{-2} day^{-1}) for Italian ryegrass (**a**) and tall fescue (**b**). *Symbols* as in Fig. 10. Linear regressions for Italian ryegrass ($y = 0.0005x + 0.907$; $R^2 = 0.59$; all treatments taken together) and tall fescue ($y = 0.0013x + 0.324$; $R^2 = 0.81$; N+/irr treatment only) are represented

The water extraction profiles for dry plots were computed under the assumption that vertical water movements within the soil and from the soil to the atmosphere were negligible. Such vertical water fluxes were indeed unlikely to occur during the dry periods that were chosen for this computation. For the same reason, direct evaporation from the dry soil surface was probably limited (Ritchie 1972). Because of that approximation, the contribution of top soil horizons was probably only slightly overestimated. Under optimal water status, the water extracted from a particular soil layer is proportional to root length density (Maertens et al. 1974; Sharp and Davies 1985). The value of the transpiration flux in each horizon is therefore only dependent on RLD and ET$_0$. Given that, by definition, ET$_0$ corresponded to the water use of a well-watered tall fescue sward, it was likely to be close to the true level of evapotranspiration in our case.

Soil nitrogen fluxes were computed assuming that average [NO$_3$] and [NH$_4$] levels were close to the values interpolated between the dates when samples were collected. Short-term variations linked to mineralization or denitrification were thus ignored. These hypotheses were likely as the periods considered were short and dry. Nor was it possible to capture the flux of nitrates produced by local nitrification and immediate absorption by roots using the approach described here. However, this flux can make a significant contribution to the nitrogen balance of grass swards (Lemaire et al. 2004). In the case of fertilized crops, and under dry conditions, this contribution of the soil mineral flux was probably very limited. But in the case of low N irrigated treatments, that omission may have been one of the factors that weakened the correlation between the two computed fluxes. Finally, and as with many other approaches, the computation assumed that [NO$_3$] and [NH$_4$] were close to nil at the root absorption surface (Hoffland et al. 1990). Although that is clearly an approximation, it is currently used in some crop simulation models such as STICS (Brisson et al. 2009) or CERES (Gabrielle et al. 1998); it indeed tended to largely overestimate the real contribution of diffusion when the N flux was larger than uptake, as was the case with Italian ryegrass. Indeed, in that case, given the figures obtained for convection (which alone could satisfy the crop N demand), the mineral N concentration at the root surface may well have been higher than the mean soil mineral N concentration. Diffusion in the opposite direction to mass flow, i.e. from the rhizosphere to the rest of the soil volume, could even be expected. Again, because diffusion only made a minor contribution, this did not significantly affect the main trend.

The relationship between the soil N flux and N uptake was more linear than the relationship between soil [NO$_3$] and N uptake established by Devienne-Baret et al. (2000). It is likely that the relationship was less consistent in wheat because of the longer period studied in that earlier work. Similarly, Farruggia et al. (2004) showed a loose correlation between soil mineral concentrations and N_{NI} in grass swards. Indeed, at least in a summer context when deeper soil horizons contributed a larger proportion of the total flux, the soil mineral flux computed in this work appeared to be more directly linked to N uptake. Some variations in the mineral N flux to roots were observed between the plots as a result of variations in transpiration rates, root biomass RLD and soil characteristics.

However, for each plot, the main driving force was clearly the ET_0. The soil mineral N concentration was not subject to major errors. By contrast, the estimated N balance in the sward was more directly affected by heterogeneity in root and herbage dry matter, the nitrogen concentration of tissues being more homogenous.

The N_{NI} values obtained confirmed that Lm benefited from an excess N supply. The coincidence between N uptake and N flux in tall fescue suggested that the species might have been under nitrogen deficiency. However, only N_{NI} very clearly supported that hypothesis. Italian ryegrass always had a nitrogen nutrition index of 1 or higher, indicating that nitrogen was never limiting for that species during this study. The fact that N_{NI} was positively and linearly correlated with the soil N flux showed that plants continued to accumulate N in excess of their current needs to ensure growth. This could be interpreted as an adaptation of plants to situations where mineral N is only available for short periods of time, when it is important that N reserves can be stored in the plant for future use (Burns 1992).

Both the slope and the intercept of the relationship between the soil N flux and N_{NI} were dependent on crop growth conditions and on the initial N_{NI} value for the period studied. A more complete analysis of plant supply and use, including the partitioning of N within the plant, will be necessary to enable the actual computation of N_{NI} from soil and climatic conditions.

Conclusion

The nitrogen nutrition status of a grass sward is dependent on water availability. This dependency was clearly explained by the dependence of N supply on the soil water flux induced by crop transpiration. Although our knowledge of soil conditions, root architecture and biomass remains limited, and despite the considerable heterogeneities encountered in soil, the simple approach adopted by crop simulation models can reflect the main effects causing drought-induced nitrogen deficiency in grass swards under summer conditions. This analysis also demonstrated the extent to which nitrogen status could increase beyond an optimum level proportionally to the supply of mineral nitrogen. Better account needs to be taken of this factor in current N uptake models in order to generate the nitrogen content of crops and enable its longer term management.

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