RESEARCH ARTICLE

Critical N concentration can vary with growth conditions in forage grasses: implications for plant N status assessment and N deficiency diagnosis

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Abstract The N nutrition index (NNI, defined as the actual shoot N concentration relative to a reference critical value, N_{ref}) is a valuable tool to analyze the N nutrition status of crops. We present empirical evidence obtained for a range of forage grass species and environmental conditions with the aim to contribute to verify the NNI as a tool to diagnose N deficiency and to examine responses to N fertilization. Ten N fertilization experiments were conducted in the SE of the Pampa region (Argentina) and included (1) perennial (*Festuca arundinacea* Schreb. *Thinoppyron ponticum* (Podp.) Barkworth and Dewey) and annual (*Lolium multiflorum* Lam., *Avena sativa* L. *Bromus catharticus* H.B.K.) grasses, (2) autumn, winter and spring re-growths, and (3) good and poor quality soils. Experiments comprised four to six N fertilization rates, each including five to seven sequential harvest dates. Plots were replicated in three or four complete randomized blocks. All experiments were rainfed and adequately provided with phosphorus. The critical N concentration (N_{cr}) -that is the minimum plant N concentration required to reach maximal accumulated shoot biomass-was determined for each harvest date. Observed N_{cr} were then compared to values predicted by the general reference curve (N_{ref}) proposed elsewhere. In six of the ten experiments measured N_{cr} agreed with predicted N_{ref}. Thus, the present study extends to marginal soils, to species adapted to such environment, and to winter growth conditions the empirical support for the use of N_{ref} in the quantification of pasture N deficiency. But N_{cr} was lower than N_{ref} in four experiments in which growth conditions were not adequate for reaching potential growth rates. Therefore, it is suggested that when factors other than N are expected to limit pasture growth, an N_{cr} lower than N_{ref} should be considered. In all experiments, a direct relationship between accumulated shoot biomass and NNI was found, radiation use efficiency (RUE) being more negatively affected than photosinthetically active radiation interception (PARi) by N deficiency. Reductions in the latter were relevant only from moderate to severe N deficiency. Notably, the NNI achieved at initial stages of regrowth (150-200°C day after the fertilization date) was highly associated with the N nutritional status along the regrowth and, therefore, confirming NNI as a promising tool for diagnostic purposes.

Keywords Critical N concentration · N fertilization · N nutrition index · Forage grasses

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Introduction

Excessive levels of N fertilization have often been used in Europe and other areas of the world to achieve pasture potential yield. Conversely, the livestock production scheme in the Argentinean Pampas has been characterized by extensive agronomic management and sub-optimal pasture production levels. However, land use intensification in the last decade as a result of the expansion of cash crops and the displacement of cattle production to less fertile soils entails severe threats to soil fertility and pastures productivity persistence. Agricultural expansion, a global phenomenon, requires for practices to reasonably match productive as well as sustainability goals through the efficient management of N in the agroecosystems.

Regardless of the N source (fertilizer, biological fixation, OM mineralization), a reasonable correspondence between N supply and crop N demand is required in order to ensure a high use efficiency of N. The crop N demand refers to an optimum N status of the crop that can be quantified by the critical N concentration, that is, the minimum N concentration in the accumulated shoot biomass that is required to attain the maximum instantaneous growth rate. The critical N concentration was not widely accepted as a general concept until the generalized non linear decline between critical N concentration and shoot biomass accumulation was revealed as a general phenomenon for vegetative crops among a wide range of species (Lemaire and Salette 1984; Greenwood et al. 1986; Greenwood et al. 1991). At present there is a strong theoretical and experimental basis to support the critical N concentration as a fundamental reference to determine the actual N nutrition status during crop growth, independently of the other environmental conditions and of genetical characteristics, when growth is not restricted by the soil N supply (e.g. Lemaire and Gastal 1997; Gastal and Lemaire 2002). It is worth noting, however, that some authors have suggested the existence of some genetic (Bélanger et al. 2001) as well as aging induced changes in tiller population structure of pastures (Bélanger and Ziadi 2008) that could affect the critical N curve. The critical N concept has provided the basis to define a N nutrition index, assessed as the ratio between the actual and the critical N concentration in the plant, which has been demonstrated to vary proportionally to

the crop growth rate (Lemaire and Gastal 1997; Plénet and Cruz 1997; Marino et al. 2004) and, hence, to the intensity of the N deficiency experienced by the crop (Gastal and Lemaire 2002).

In the present paper we present results from a series of experiments carried out during several years, in both annual and perennial forage grasses growing under a wide range of edaphic and climatic conditions in the SE of the Argentinean Pampas with the aim to confirm the relevance of NNI on other species and other agroecological conditions, as proposed by Lemaire and Gastal (1997), as a tool to examine responses to N fertilization. For this purpose, we first check the generality of the proposed reference dilution curve of N concentration (onwards N_{ref}) by comparing the critical N concentration determined with the statistical approach proposed by Justes et al. (1994) (onwards N_{cr}) with the corresponding value estimated according to the empirical relationship proposed by Lemaire and Salette (1984). Second, we examine the association between plant N status, as quantified by its NNI, and the accumulated shoot biomass and its determinants, photosynthetically active radiation interception (PARi) and radiation use efficiency (RUE). Finally, we analyze the use of NNI as a tool for the prediction of responses to N fertilization at early stages of the regrowth.

Materials and methods

Ten experiments were conducted on perennial (*Festuca arundinacea* Schreb. and *Thinoppyron ponticum* (Podp.) Barkworth and Dewey) and annual (*Lolium multiflorum* Lam., *Avena sativa* L. and *Bromus catharticus* H.B.K.) species established in pastures in the southeast of Buenos Aires Province, Argentina (Balcarce, 37°45′S 58°18′W, and Tandil, 37°14′S 59°15′W). Soil and climatic characteristics and management conditions for each experiment are summarized in Tables 1 and 2, for perennial and annual pastures, respectively. Phosphorus Bray I values were higher than the minimum critical threshold of 12–15 mg P kg^{-1} soil below which pastures respond to P fertilization in this region (Echeverría and García 1998; Marino and Berardo 2000). It is worth mentioning that under natural field conditions low availability of P (García 2001, 2003) and non limiting K availability (Moscatelli et al. 2009) are common

Table 1 General characteristics and management conditions for perennial species experiments

Experiment		TF MK96[a] and TF EP96[b]	TW99a and TW99lw
Species		*Festuca arundinacea*	*Thinopyrum ponticum*
Cultivar		Maris Kasba[a]	El vizcachero
		El Palenque[b]	
Year of establishment		1982	
Pasture condition		Excellent	Excellent
Location		Balcarce	Balcarce
Soil classification[c]		Typic Natraquoll	Typic Natraquoll
Initial soil P content (mg kg^{-1}, Bray I)		na	31.2
Soil pH (water)		7.0	9.4
		Autumn (TW99a)	Late winter (TW99lw)
Experimental period	20 May 1996–24 Set 1996	30 Mar 1999–17 Jun 1999	20 Sep 1999–16 Nov 1999
Rainfall (mm)	218.2	98.4	107.5
Mean temperature (°C)	8.9	11.3	13.6
N fertilization dates	20 May	30 Mar	20 Sep
N Applied (kg ha^{-1})	0, 50, 100, 150, 200	0, 90, 180, 270	0, 150, 300
P fertilization dates	25 Apr	30 Mar	20 Sep
P Applied (kg ha^{-1})	30	30	30

Soil P content and pH correspond to the 0-100 mm depth layer

na data not available

[c] Soil characteristics are described in Buol et al. (1989)

characteristics of the soil types where the experiments were carried out.

Experimental management

The corresponding rates of N and P fertilizer (Tables 1 and 2), applied as urea and triple superphosphate, were surface-broadcasted following an initial cut to 50 mm cutting height. Subsequently, 4–6 harvests (depending on the experiment) to 50 mm height were carried out every 7–10 days on independent plots of 5.5 m^2 using a motor mower. The duration of the regrowths were in the range of 539–556°C day for the temperate tall fescue and for tall wheatgrass, 620°C day for the Mediterranean tall fescue and 598–676°C day for the annual grasses (base temperatures of 4°C for the mediterranean tall fescue—estimated from Lattanzi 1998, 4.5°C for the temperate tall fescue and tall wheatgrass—estimated from Lattanzi 1998 and Borrajo 1998, respectively, and 0°C for the annual grasses were considered). The durations roughly coincide with the expected leaf life spans of the studied species (Lemaire and Agnusdei 2000; Agnusdei et al. 2001) and, accordingly, non

significant losses through senescence were supposed to occur during the experimental periods.

Determinations

The material harvested on each plot was weighed (fresh weight) and one sub-sample (300-500 g fresh weight) was oven dried at 60°C for 48 h to determine the dry matter content in order to estimate shoot biomass accumulation (Mg DM ha^{-1}). The sub-samples corresponding to the three replicates (blocks) of the same treatment and harvest date were pooled and ground (1 mm mesh) to determine total N concentration in shoot biomass (N_{act}) according to Nelson and Sommers (1973, Method A without salicylic acid modification).

Radiation measurements were made at midday every 10 days from the initial cut using a tube solarimeter type TSL (Delta-T Devices Ltd., Burwell, Cambridge, UK). The proportion of radiation intercepted by the canopy (%IR) was estimated as follows:

$$\%IR = 100[(1 - IRb)/IRa]$$

where IRb denotes the average of three below canopy measurements of non intercepted global radiation and

Table 2 General characteristics and management conditions for perennial species experiments

Experiment	AR94	AR95[a] and O95[b]	AR97	AR03	PG97
Species	*Lolium multiflorum*	*Lolium multiflorum*[a] *Avena sativa*[b]	2*Lolium multiflorum*	*Lolium multiflorum*	*Bromus catharticus*
Cultivar	Grasslands Tama	Grasslands Tama	Grasslands Tama	Grasslands Tama	Martín Fierro
Sowing date	3 Mach 1994	14 March 1995	4 March 1997	5 March 2003	4 March 1997
Crop condition	Excellent	Excellent	Excellent	Excellent	Excellent
Location	Tandil	Balcarce	Balcarce	Balcarce	Balcarce
Previous crop	Perennial pasture	Perennial pasture	Perennial pasture	Perennial pasture	Perennial pasture
Soil classification[c]	Typic Argiudoll	Typic Argiudoll	Typic Argiudoll	Typic Argiudoll	Typic Argiudoll
OM (g kg^{-1})	55	57	53	77	53
Initial soil P content (mg kg^{-1}, Bray I)	18.0	13.3	19.8	21.5	19.8
Soil pH (water)	na	na	na	6.4	na
Experimental period	11 Aug 1994–11 Oct 1994	8 Jul 1995–9 Oct 1995	9 Aug 1997–9 Oct 1997	6 May 2003–2 Jul 2003	9 Aug 1997–9 Oct 1997
Rainfall (mm)	67.5	69.2 + 140.0 irrigation	126.0	68.5	126.0
Mean temperature (°C)	10	9.7	10.8	10.4	10.8
N fertilization dates	11 Aug 1994	8 Jul 1995	9 Aug 1997	6 May 2003	9 Aug 1997
N Applied (kg ha^{-1})	0, 50, 100, 150, 200,250	0, 50, 100, 150, 200,250	0, 50, 100, 150, 250	0, 40, 120	0, 50, 100, 150, 200,250
P fertilization dates	11 Aug 1994	8 Jul 1995	9 Aug 1997	6 May 2003	9 Aug 1997
P Applied (kg ha^{-1})	50	50	30	30	30

Soil P content and pH correspond to the 0-100 mm depth layer

na data not available

[c] Soil characteristics are described in Buol et al. (1989)

IRa the global incident radiation. Daily %IR was estimated by interpolation using a linear regression between the registered %IR and the number of days between two successive measurements. Photosynthetically active radiation (PAR) was calculated by multiplying the daily global incident radiation (MJ m^{-2} day^{-1}) by 0.48 (Gosse et al. 1986). In turn, daily intercepted PAR (PARi) was obtained by multiplying daily PAR by the corresponding %IR.

The radiation use efficiency (RUE, g DM MJ^{-1}) was estimated as the slope of the linear regression, forced through the origin, between cumulative shoot biomass and cumulative PARi (Gosse et al. 1986). Intercepted PAR and RUE were not determined in experiments AR94 and TW99lw.

For each experiment the procedure proposed by Justes et al. (1994) was used as a statistical tool to estimate critical N values ($N_{cr} = a\ SB^{-b}$, where SB indicates shoot biomass, Mg DM ha^2). In order to do

this, for each sampling date, data of shoot biomass under the different N levels were analyzed by ANOVA. The minimum shoot biomass value not significantly different ($P = 0.10$) from the maximum was identified. The average maximum shoot biomass between not significantly different data points was then calculated. The critical N concentration corresponded to the ordinate of the intersection point of the oblique line of joined increases in shoot biomass and N concentration and the vertical line corresponding to the average maximum shoot biomass value. Finally, only estimated shoot biomass values equal or higher than 1 Mg DM ha^{-1} were considered since non significant changes in N concentration of aerial parts are expected below this level of accumulated DM.

The NNI was calculated as the ratio between N_{act} and N_{ref}. Relative shoot biomass (Shoot:Shoot$_{max}$), relative PAR$_i$ (PARi:PARi$_{mac}$) and relative RUE (RUE:RUE$_{max}$) were calculated as the ratio between

the value of the variable for each N treatment and the corresponding maximum value. Relative variables were plotted vs. weighted NNI (NNI_w). The NNI_w was calculated as the time-weighed average of the instantaneous NNI (i.e. NNI estimated at each sampling date) (Lemaire and Gastal 1997). For this purpose, the average instantaneous NNI corresponding to each pair of successive sampling dates were weighed by the corresponding time interval, resulting values being then added and divided by the total length of the regrowth period. When the critical N dilution curve was significantly different from the reference N dilution curve, the NNIs used for the calculation of the NNI_w were estimated as the ratio between N_{act} and N_{cr}.

The critical N uptake of the pastures was calculated according to Lemaire and Gastal (1997) as follows:

$$N_{uptake} = 10a(SB)^{(1-b)}$$

where 10a corresponds to the quantity of N taken up by the sward when shoot biomass (SB) reaches 1 Mg DM ha^{-1}, and the coefficient 1-b represents the ratio of the relative rate of N accumulation (dN/Ndt) to the relative rate of shoot biomass accumulation (dSB/SBdt).

Experimental design and statistical analysis

The experimental design was a split-plot with three randomized complete blocks. Harvest dates were main plots and N treatments were subplots (1.0 m × 5.5 m). Data analyses were performed using the SAS system for Windows version 9.0 (SAS Institute, Cary, NC, USA). Shoot biomass data were tested by LSD ($P = 0.10$) after ANOVA using the GLM procedure. Critical N curves were fitted using non linear regression models (NLIN procedure). In order to statistically compare the N_{cr} curves of each experiment with the N_{ref} curve (Lemaire and Salette 1984, $N_{ref} = 4.8$ SB$^{-0.32}$), linear regressions were adjusted to the log-transformed shoot biomass values estimated for each sampling date using Justes' procedure (see explanation above) and the resultant log-transformed shoot biomass N concentration estimated from both curves. Slopes were statistically analyzed using the program (S)MATR (http://www.bio.mq.edu.au/ecology/SMATR), version 2.0 (Falster et al. 2006).
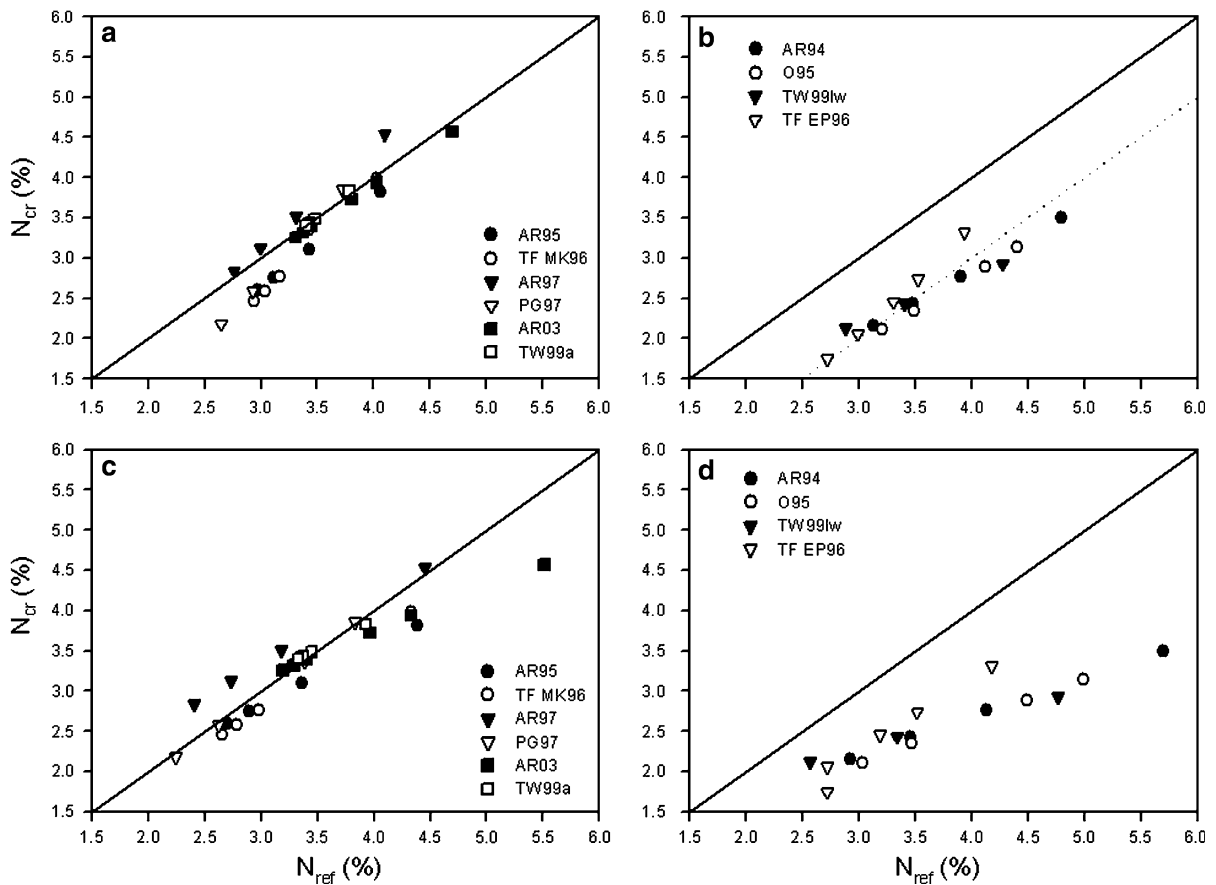
Results

Critical N concentration

In six out of the ten experiments, non significant differences ($P > 0.05$) between the slopes of the log-transformed critical and reference N dilution curves were detected. Accordingly, critical N concentration values (N_{cr}), determined from our data according to the procedure proposed by Justes et al. (1994), agreed with those predicted by the reference curve (N_{ref}) 4.8 W$^{-0.32}$ proposed by Lemaire and Salette (1984) (Fig. 1a). The agreement was particularly close for values higher than 3.2%, corresponding to shoot biomass lesser than 3.2 Mg ha^{-1}. In the other four experiments significant differences ($P < 0.05$) between the corresponding slopes were detected and N_{cr} did not match the expected N concentration according to the reference curve above (Table 3) and were around 1 percentage unit lower than N_{ref} (Fig. 1b). Therefore, as it will be addressed later, in these specific cases the N_{ref} overestimated the minimal concentration of N required for maximal growth.

Since there is some discussion on the estimation of the reference critical N concentration, and suggestions that it might depend on crop species (cf. Greenwood et al. 1990; Lemaire and Gastal 1997; Justes et al. 1994), we compared the N_{cr} determined applying Justes et al. (1994) procedure with the corresponding values predicted by the reference curve 5.7 SB$^{-0.50}$ proposed by Greenwood et al. (1990) for C3 species. In this comparison, the deviation observed in the first six experiments between N_{cr} and N_{ref} values lower than 3.2% disappeared (Fig. 1c). However, the lack of fit observed in the other four experiments still remained. This result cannot be attributed to the divergence between the critical N concentrations estimated according to Lemaire and Salette (1984) or to Greenwood et al. (1990), but to an actual feature of the referred datasets.

Estimation of the nutritional status of crops: the N nutrition index (NNI)

Pasture N status was quantified with the NNI, estimated as the ratio of actual N concentration to N_{ref} (Figs. 2 and 3). In the case of the four experiments where Ncr did not match Nref (Fig. 3), the relative

Fig. 1 Relationships between the critical N concentration values according to: **a** and **b** Lemaire and Salette (1984), $N_{ref} = 4.8\ SB^{-0.32}$ (SB = shoot biomass), or **c** and **d** Greenwood et al. (1990), $5.7\ SB^{-0.50}$, indicated as N_{ref}, and the critical N concentration values calculated according to Justes et al. (1994) procedure (N_{cr}) for ten different fertilization field experiments. **a** and **c** Experiments were N_{cr} was similar to N_{ref}; **b** and **d** experiments where N_{cr} differed form N_{ref}. The *dotted line* in **b** illustrates de average departure from the 1:1 line

Table 3 Critical N curves adjusted according to Justes et al. (1994) for the four experiments in which the critical N concentratio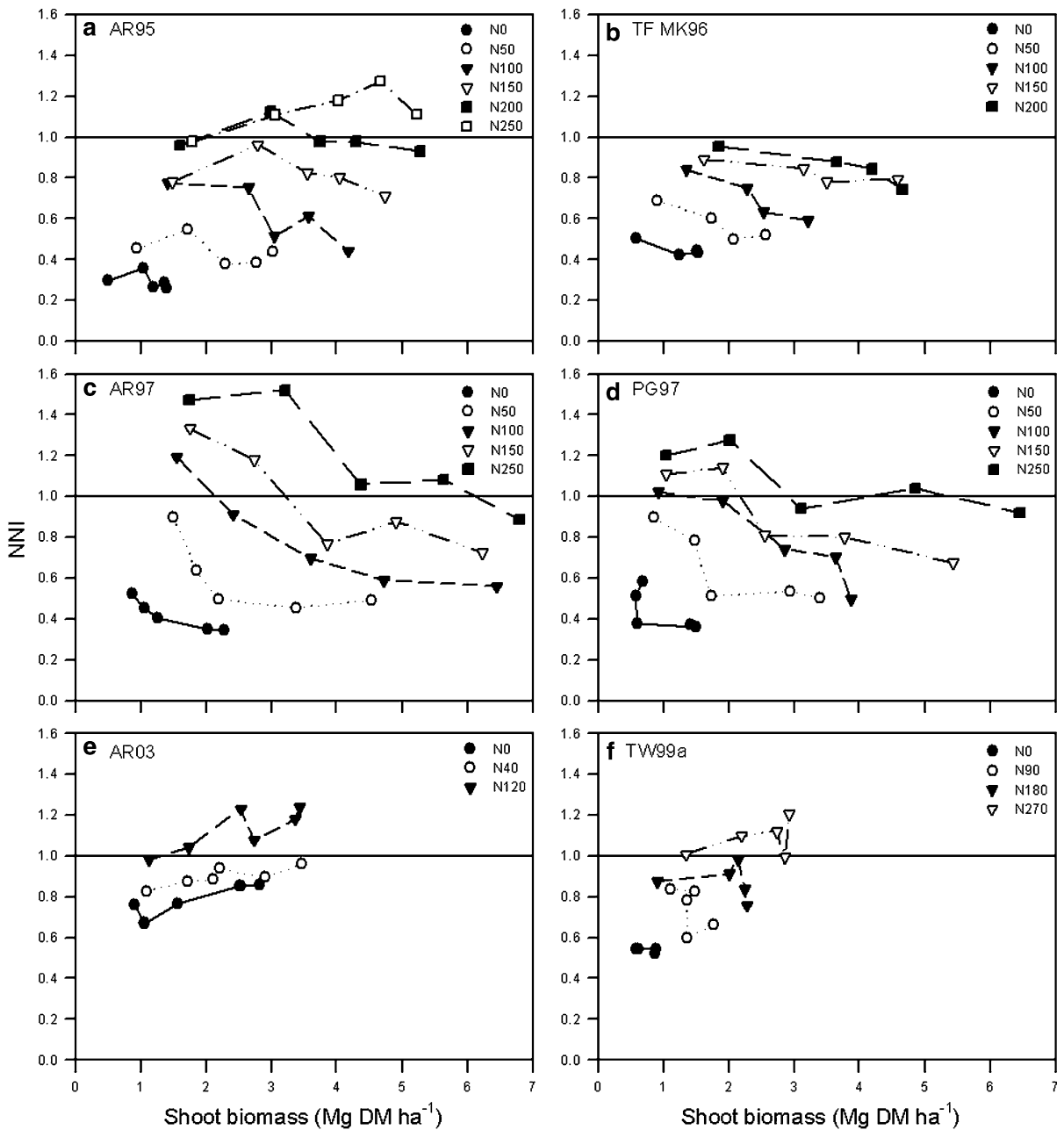n did not match the reference N value estimated according to Lemaire and Salette (1984), and the corresponding range of shoot biomass (SB) for which they were determined

Experiment	Adjusted critical curve	Actual shoot biomass range (Mg DM ha^{-1})
AR94	$N_{cr} = 3.5SB^{-0.36}\ R^2 = 0.90$	1.0–4.8
O95	$N_{cr} = 3.5SB^{-0.40}\ R^2 = 0.54$	1.3–3.4
TW99w	$N_{cr} = 3.2SB^{-0.26}\ R^2 = 0.84$	0.4[a]–5.0
TF EP96	$N_{cr} = 4.7SB^{-0.55}\ R^2 = 0.98$	0.7[a]–3.2

[a] Only values above 1 Mg DM ha^{-1} were used to adjust the critical curves (according to Lemaire and Gastal 1997)

position of the different points in relation to the N_{cr} is indicated through a dotted line that represents the $N_{cr}:N_{ref}$ ratio. Results indicate that using N_{ref} in the calculation of the NNI yielded a lower plant N status estimation than using N_{cr}.

During late-winter/early-spring, the N status of the pastures tended to decrease during the regrowth (Fig. 2a-d), indicating an accentuation of N deficit, and therefore, a progressive greater departure from the reference N uptake curve (Fig. 4). On the
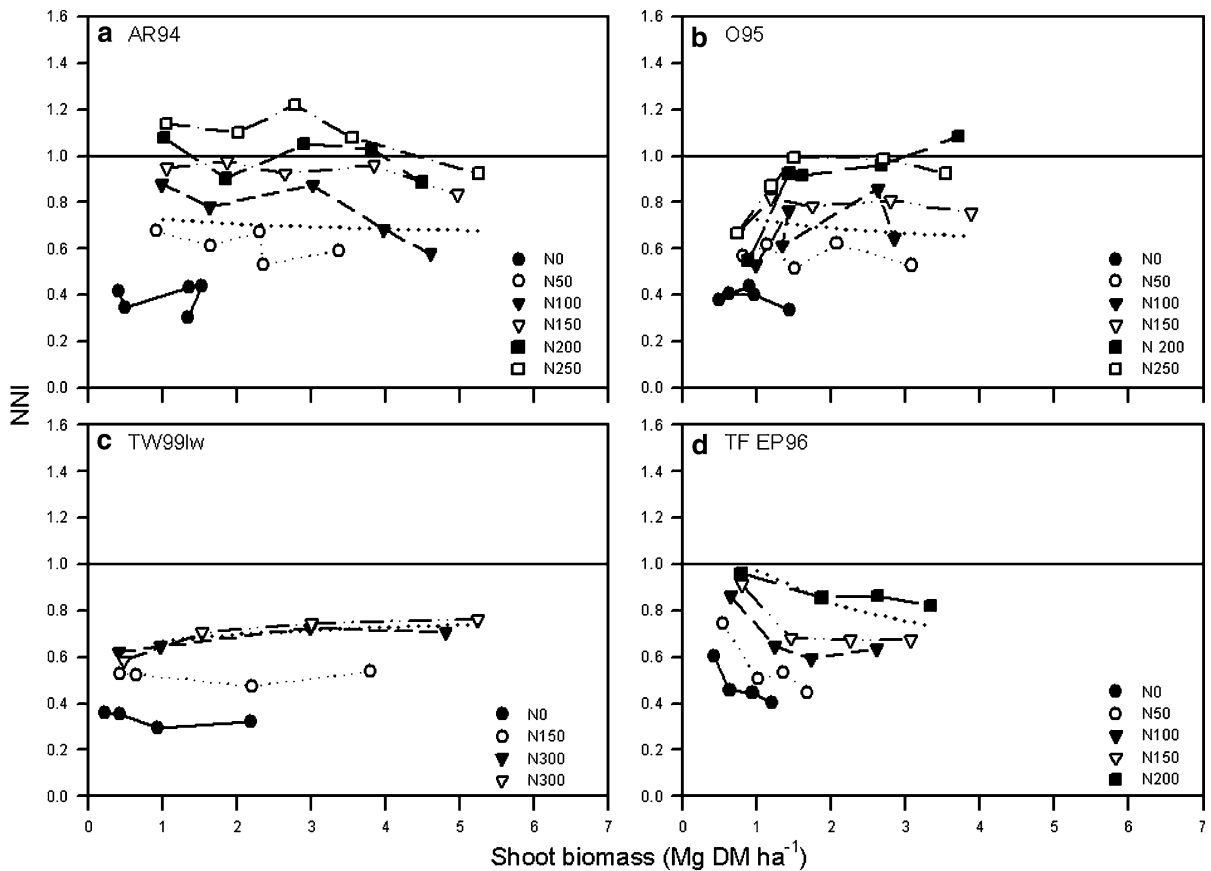
Fig. 2 Evolution of the instantaneous N nutrition index (NNI) in relation to accumulated shoot biomass in six fertilization field experiments where the critical N concentration [N_{cr}, calculated according to the procedure proposed by Justes et al. 1994) was similar to the reference N concentration (N_{ref} = 4.8 SB$^{-0.32}$, Lemaire and Salette 1984)]. The NNI were calculated as the ratio between actual N concentration and N_{ref}

contrary, in autumn, crop N status remained comparatively more stable during the regrowth, or even tended to increase (Fig. 2e-f). In these cases, pasture N uptake followed a trajectory more or less parallel to the reference curve, except in the tall wheatgrass experiment (TW99a) in which young leaves in plants of high N-rate treatments died due to freezing temperatures, this leading to a sharp decrease in shoot N accumulation in the last two sampling dates (Fig. 4).

Fig. 3 Evolution of the instantaneous N nutrition index (NNI) in relation to accumulated shoot biomass in four fertilization field experiments where the critical N concentration (N_{cr}, calculated according to the procedure proposed by Justes et al. 1994) differed from the reference N concentration ($N_{ref} = 4.8$ $SB^{-0.32}$, Lemaire and Salette 1984). The NNI were calculated as the ratio between the actual N concentration and N_{ref}. The *dotted line* represents the $N_{cr}:N_{ref}$ ratio

Relationship between crop nutritional status and crop yield components (PAR_{int}, RUE)

In the experiments where Ncr matched Nref, results confirmed the existence of a direct association between pasture production and N nutrition: the relative reduction in crop yield (Shoot biomass/Shoot biomass$_{max}$) was closely related to the crop nutritional status as measured by its weighted NNI (NNIw, Fig. 5), (Lemaire and Gastal 1997). Most notably, the same linear association between relative yield and NNIw was also verified in experiments carried out under limiting growth conditions where the observed N_{cr} (instead of N_{ref}) was used to estimate the NNI (Fig. 6). In both cases, the NNI was a good indicator of the expected productive performance of the pasture.
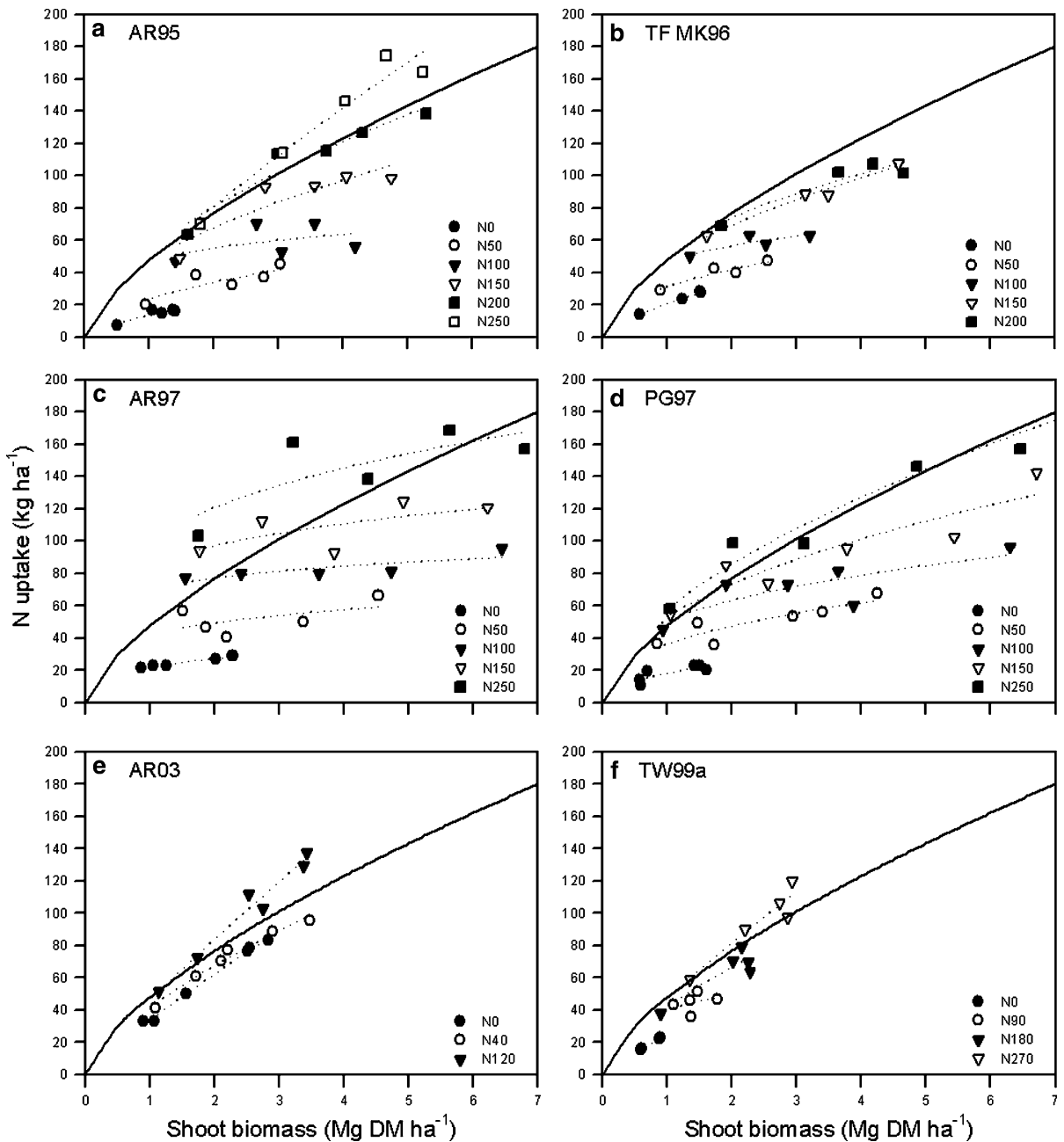
Radiation use efficiency (RUE) diminished under N deficiency in both groups of experiments (Figs. 5 and 6), the reduction in RUE being roughly proportional to that in NNI_w. Conversely, light to moderate N deficiency had little effect on the amount of intercepted PAR (PARi). But at NNIw values lower than 0.80, a sharp decrease in PARi occurred in all experiments (Figs. 5 and 6).

Discussion

On the generality of the reference curve of critical N concentration

A general stable allometric relationship describing a minimum (critical) N uptake required for maximum
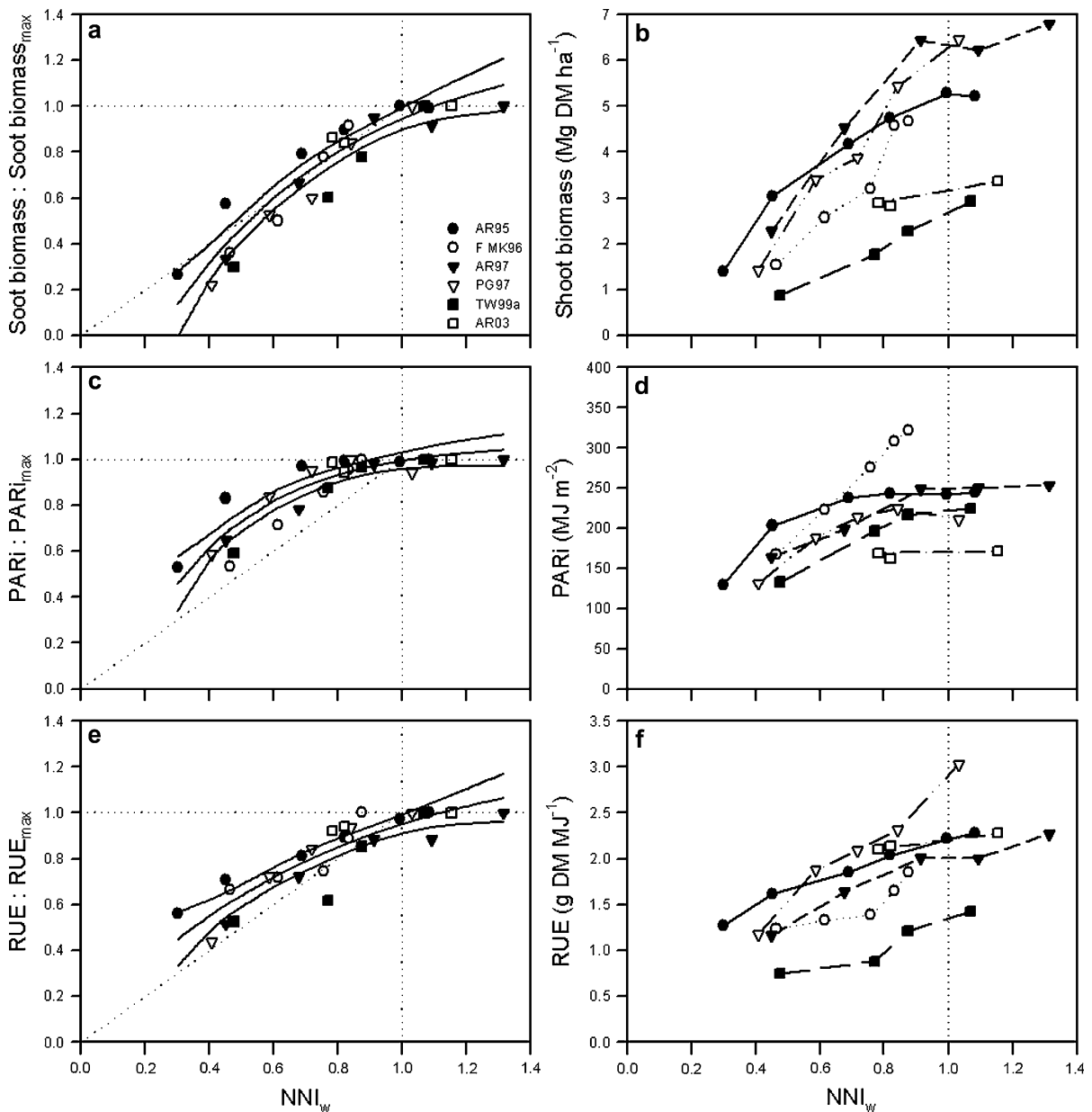
Fig. 4 Evolution of shoot N uptake in relation to shoot biomass accumulation (SB) in six fertilization field experiments where N_{cr} was similar to N_{ref}. The *filled line* indicates N uptake = 48 SB$^{0.68}$. The different *dotted lines* indicate the evolution of N uptake in each fertilization treatment

shoot biomass accumulation in vegetative grass canopies implies the existence of a reference curve that describes the minimum (critical) plant N concentration allowing maximum crop growth (Lemaire and Salette 1984; Greenwood et al. 1990, 1991). Theoretical support for this concept derived from

growth models that distinguish metabolic from structural plant components and from N recycling from basal, shaded, old leaves towards well-lit upper strata of plant canopies, is given in Greenwood et al. (1991) and Lemaire and Gastal (1997). In six out of the ten experiments reported in the present study, N_{cr}
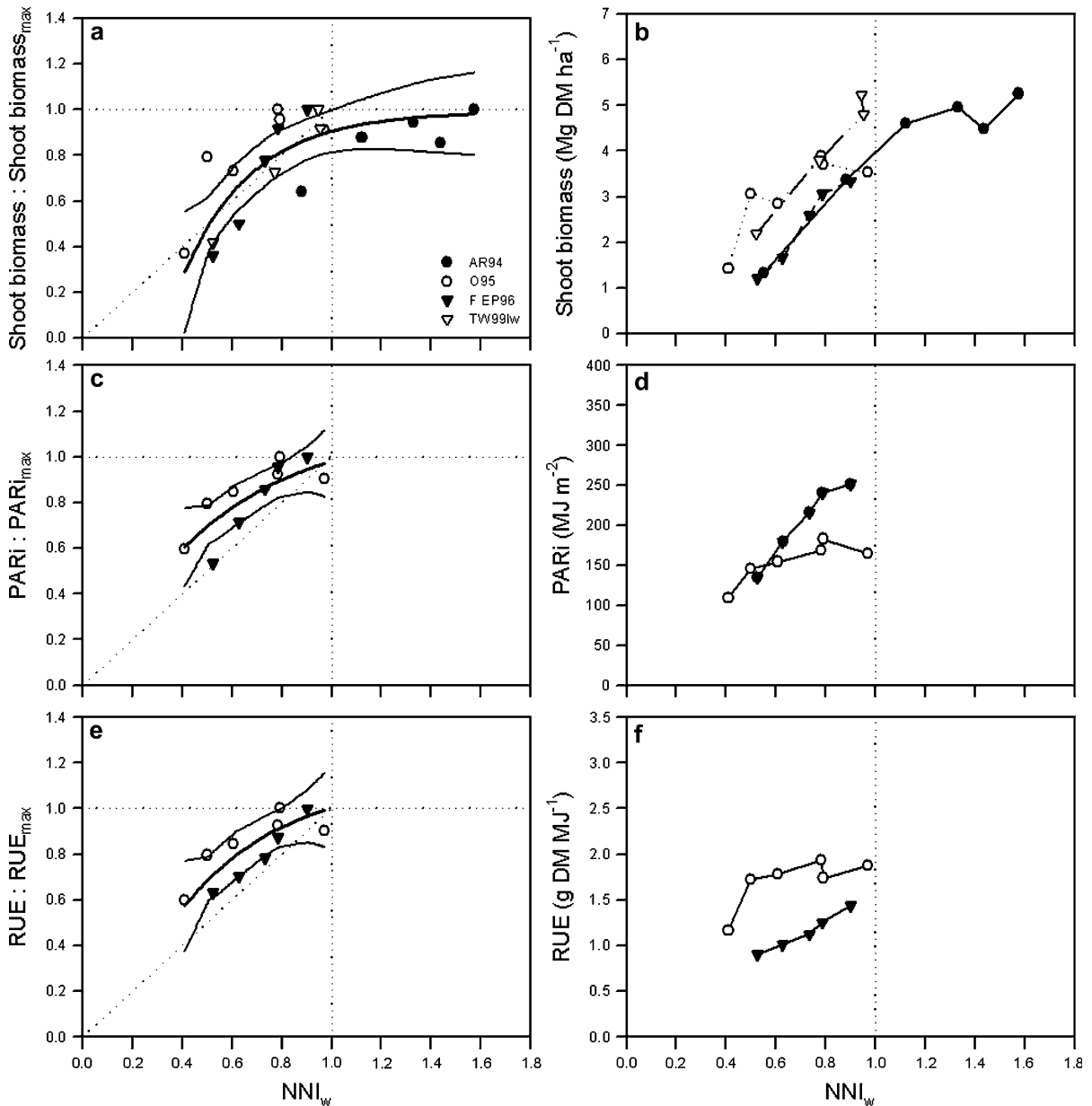
Fig. 5 Relationships between the relative shoot biomass accumulated at the last harvest date (Shoot biomass:Shoot biomass$_{max}$, **a** the relative PARi (PARi:PARi$_{max}$, (**c**) and the relative RUE (RUE:RUE$_{max}$, (**e**) and the N nutrition index weighed as a function of time (NNI$_w$). *Lines* represent the monomolecular equations [Shoot biomass:Shoot biomass$_{max}$ = 1.30 (1-1.48 exp(−1.68 NNI)), PARi:PARi$_{max}$ = 1.07 (1-1.39 exp(−2.97 NNI)), RUE:RUE$_{max}$ = 1.30 (1-0.96 exp(−1.28 NNI))] and its 95% confidence interval (according to Bélanger and Richards 1997). The evolution of accumulated shoot biomass (**b**), PARi (**d**) and RUE (**f**) with the NNIw in six fertilization field experiments where N$_{cr}$ was similar to N$_{ref}$ are further presented

measured on various annual and perennial C3 grasses growing under different soil types agreed closely with predicted N$_{ref}$ values, thus expanding the body of data that empirically support the idea that N$_{ref}$ could be considered a general biological characteristic of plant canopies.

Slightly different N$_{ref}$ curves coexist in the literature, depending mainly on the species considered (see

Fig. 6 Relationships between the N nutrition index weighed as a function of time (NNI_w) and the relative shoot biomass accumulated at the last harvest date (Shoot biomass:Shoot biomass$_{max}$), the relative PARi (PARi:PARi$_{max}$) and the relative RUE (RUE:RUE$_{max}$) in four fertilization field experiments where N_{cr} differed from N_{ref}. *Lines* represent the monomolecular equations [Shoot biomass:Shoot biomass$_{max}$ = 0.99 (1-3.02 exp(−3.55 NNI)), PARi:PARi$_{max}$ = 1.14 (1-1.34 exp(−2.43 NNI)), RUE:RUE$_{max}$ = 1.14 (1-1.09 exp(−2.05 NNI))] and its 95% confidence interval (according to Bélanger and Richards 1997). The evolution of accumulated shoot biomass (**b**), PARi (**d**) and RUE (**f**) with the NNIw in six fertilization field experiments where N_{cr} was similar to N_{ref} are further presented

review in Gastal and Lemaire 2002), but also on the procedure used to estimate the critical N concentration (cf. Justes et al. 1994; Plénet and Cruz 1997; Greenwood et al. 1990). In the present study, the agreement between observed N_{cr} and predicted N_{ref}

improved when the latter was calculated as 5.7 SB$^{-0.50}$, according to Greenwood et al. (1990), instead of the 4.8 SB$^{-0.32}$ proposed by Lemaire and Salette (1984). In our view, the magnitude of this improvement was not enough so as to ascribe it to
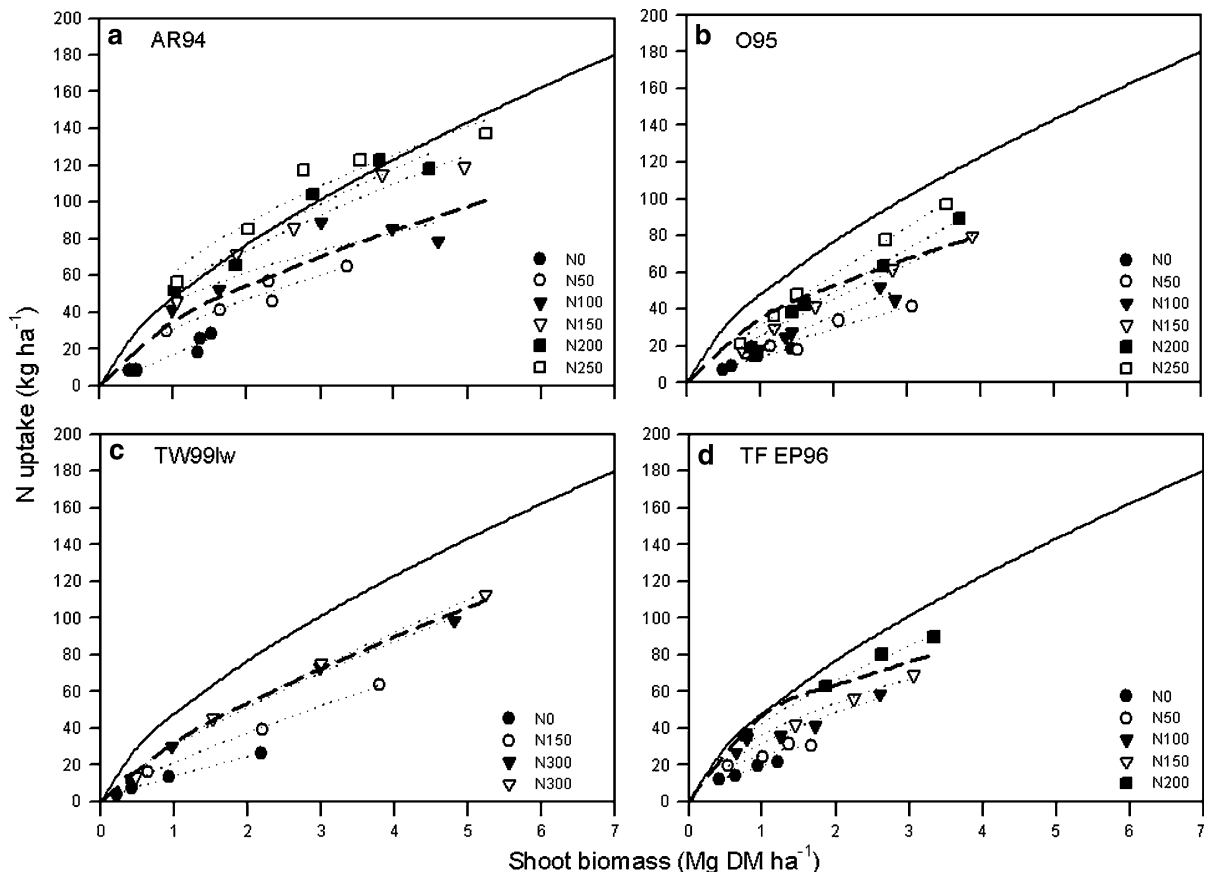
an inherent difference between these N reference curves.

In these six experiments, N availability was the major factor controlling growth, for the availability of other nutrients (especially phosphorus) and water were appropriate, and temperatures were not too low (Tables 1 and 2). Under these conditions, similar to those in which the empirical relationship between N concentration and shoot biomass accumulation was derived, our results indicate that reference curves can be used for N diagnose purposes, as proposed in Lemaire and Gastal (1997).

However, in the other four experiments observed N_{cr} were consistently lower than N_{ref} values (Fig. 1b), that is, maximal shoot biomass accumulation occurred at N concentrations lower than those predicted by N_{ref}. This discrepancy was not associated with the chosen

reference curve, as the departure from the 1:1 line was not markedly altered when different N_{ref} were used (Fig. 1b, d). Neither was it related to a lack of ability of plants to absorb N: except for TW99a, N uptake continued to increase with higher N fertilization rates, but no significant increases in shoot biomass were evident (Fig. 7).

The reason why N_{cr} was lower than N_{ref} in these four experiments is not clear. Although in potato it has been found that cultivars can affect N_{ref} (Bélanger et al. 2001), such an effect has not been reported in forage species (review in Greenwood et al. 1991; Lemaire and Gastal 1997). Further, genotypic differences are an unlikely cause in the present study: in three of the experiments (AR94, TW99lw and TF EP96) the same cultivar matched N_{ref} in other experiments (AR95, AR97, AR03, TW99a and unpublished data on tall
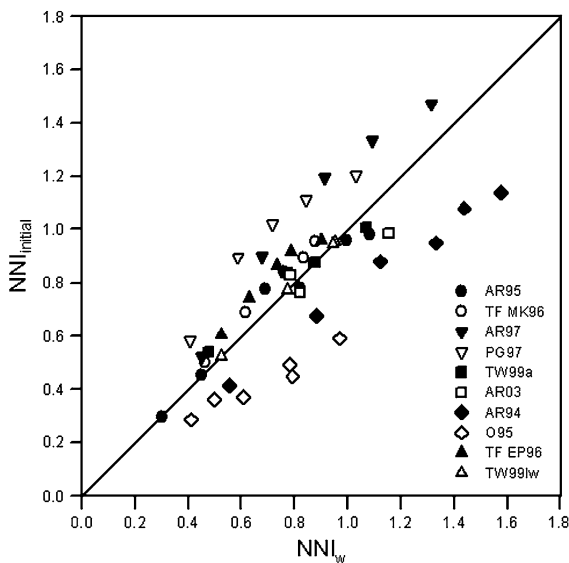

Fig. 7 Evolution of shoot N uptake in relation to shoot biomass (SB) accumulation in four fertilization field experiments where N_{cr} differed from N_{ref}. The *solid line* indicates N uptake = 48 $SB^{0.68}$. The short *dashed line* indicates N uptake calculated from the corresponding N_{cr}. Curve (N uptake$_{cr}$). The different *dotted lines* indicate the evolution of N uptake in each fertilization treatment. **a** AR94 N uptake$_{cr}$ = 37.5 $SB^{0.73}$. **b** O95 N uptake$_{cr}$ = 33.6 $SB^{0.72}$. **c** TW99lw N uptake$_{cr}$ = 29.9 $SB^{0.73}$. **d** TF EP96 N uptake$_{cr}$ = 43.1 $SB^{0.54}$

fescue cv El palenque, respectively). Neither soil type appears to be a likely reason: in the experiments carried out in marginal soils (sodic, high pH) N_{cr} matched N_{ref} (e.g. TW99a, TF MK96).

Notably, in three of these four experiments maximal growth rates (calculated as the ratio between the accumulated shoot biomass at the highest N rates and the accumulated thermal time set at around 550°C day^{-1} for all the experiments—i.e. 547 ± 11°C day^{-1}) were low relative to expected ones for the species and/ or season. Thus, TF EP96 and O95 maximal growth rates (0.60 ± 0.02 and 0.47 ± 0.06 g DM °C day^{-1}) were 20–40% lower than those achieved by companion experiments matching the N_{ref} curve, TF MK96 and AR95 (0.78 ± 0.08 and 0.82 ± 0.09 g DM °C day^{-1}), respectively. In TF EP96 winter temperatures restricted shoot growth (low RUE and PARi, Lattanzi 1998). In O95 freezing temperatures lead to plant damage and reduced tiller density (Marino 1996). In AR94, the maximal growth rate (0.66 ± 0.09 g DM °C day^{-1}) was 20–35% lower than that of the same genotype in AR95 and AR97 (0.82 ± 0.09 and 0.99 ± 0.14 g DM °C day^{-1}), respectively. In this case the precipitation during the regrowth period was relatively low (Table 2; Instituto Nacional de Tecnología Agropecuaria 2009) which suggests that climatic conditions could have limited plant growth.

Fig. 8 Comparison between N nutrition index weighed as a function of time (NNI$_w$) and the instantaneous NNI estimated at initial stages of regrowth (NNI$_{initial}$) observed in ten fertilization field experiments

Opposite to the three experiments referred above, TW99lw showed relatively high maximal growth rates (0.90 g DM °C day^{-1}), similar to those observed in the late winter regrowths where N_{cr} matched the N_{ref} (e.g. AR95, AR97 and TF MK96). This experiment is particular in the fact that an increase of 150 kg N ha^{-1} did not induce changes in shoot N concentration (Table 3). The fact that the discrepancy occurred in late winter but not in autumn might indicate an effect of season, although it would have to be an effect restricted to this species, as it was not observed in other winter regrowths.

The present study shows discrepancies between N_{cr} and N_{ref} under conditions limiting growth. Yet it is not the first that questions whether limiting growing factors other than N, such as water or nutrient deficits, could affect the relationship between N_{cr} and shoot biomass (e.g. Duru et al. 1997). Some available dataset concur with our results in suggesting that they would. For example, under low phosphorus (Duru and Ducrocq 1996) and under water deficit (Lemaire and Denoix 1987; Bélanger et al. 2001), maximal accumulation of shoot biomass occurred at N concentration lower than N_{ref}. A corollary of these results is that when growth is limited by factors other than N, the use of N_{ref} in the estimation of NNI might lead to an overestimation of the actual magnitude of N deficiency.

To elucidate this would require further research to analyze the existence of interactions between N nutrition and other growth factors (e.g. water deficit, phosphorus nutrition) on the stability of the N_{ref} curve. Since in most grasslands the existence of multiple restrictions to growth is the rule rather than the exception, and these might often be not identifiable or not susceptible to be removed through management (e.g. waterlogging, irremovable soil constraints, water deficits where irrigation is not an option, nutrient deficiencies whose correction is not economical), such studies leading to a more robust basis for the estimation of NNI would be extremely valuable.

Use of the NNI for the analysis of N fertilization results

In all ten experiments, there was a direct relationship between relative accumulated shoot biomass and N nutritional status of the pasture as quantified by the NNI. Noteworthy, some data fall below the 1:1 line suggesting that in some sub-optimal N conditions

additional factors could have also co-limited pasture yield (e.g. differences in sward structure associated to differences in pasture age, as conjectured by Bélanger and Ziadi 2008).

The analysis of N deficiency effects on two ecophysiological determinants of plant growth—the amount of PAR intercepted and the efficiency with which PAR was used to produce shoot biomass (RUE)—reveals that the effect of N deficiency upon RUE played a predominant role in explaining reduction in shoot biomass when considering the whole regrowth period. Reductions in PARi were relevant only from moderate to severe N deficiency. It should be noticed, however, that in earlier stages of regrowth intercepted PAR is more dependant on LAI, and in that case an important effect of LAI and intercepted PAR on biomass accumulation could be expected. Our results seem to contradict the relatively lesser effect that N deficiency exerts on gross and net photosynthetic carbon assimilation, both in cut (Gastal et al. 1992) and continuously grazed swards (Mazzanti et al. 1994. However, RUE comprises not only carbon capture but also its allocation between shoot and roots. Therefore, the observed effect of N deficiency upon RUE suggests a major effect of N deficiency on the partitioning of assimilates in all tested species, soil types, and seasons. This agrees with the conclusions of a study of effects of N deficiency on the components of RUE in tall fescue swards (Bélanger et al. 1992; Bélanger and Richards 1997).

Some practical implications

Early quantification of N deficiency

One of the major practical issues concerning the utilization of the NNI for pasture management is the possibility to perform an early diagnosis of the extent of N deficiency. Two key aspects to be considered are the sensitivity of the index to detect N deficiencies at the initial stages of pasture regrowth and how such initial N status is associated with pasture performance throughout the whole regrowth period. The relatively close correspondence observed between the initial NNI and NNI_w in the whole set of experiments (Fig. 8) suggests the initial NNI has a promising potential for early diagnosis of N deficiency and fertilization decision making. Moreover, considering NNI could eventually be estimated directly by remote sensing, thus by-passing the need for time consuming

determinations of shoot biomass and N content (Lemaire et al. 2008), early diagnosis would help minimize two problems associated with remote sensing: the decreasing accuracy of the estimation of shoot biomass as values increases, and the wider difference in N concentration (and thus greenness) for a given NNI at low relative to high shoot biomass values.

Autumn vs late-winter/early-spring N dressing

In the late winter regrowths optimal N status was achieved at N dressings of 200–250 kg ha^{-1} (Fig. 2a-d). These fertilization levels, which correspond to the N fertilization rate required to attain potential shoot biomass yield, were around 70% higher than those reported for temperate forage grasses (cf. Lemaire and Gastal 1997; Bélanger et al. 2008). The reasons of this difference might be associated, at least in part, to edaphic aspects related to a higher proportion of the added N being immobilized by soil microorganisms in our soils (Kuzyakov et al. 2000). From an agronomical and environmental perspective, it is worth noting that reasonable shoot biomass yields were obtained in the late winter regrowths with N rates similar those quoted in the literature for reaching potential growth (around 100-150 kg N ha^{-1}).

Another possible explanation to the generally high N rates required to attain maximum shoot biomass yields is the existence of high losses of applied N. Both the climatic conditions following fertilizer applications (mild temperatures, moderate rainfall events) and soil characteristics (slow drainage, high OM and CIC) indicate that important N looses were unlikely in both tall fescue experiments (TF EP96 and TF MK96). This was confirmed in a complementary experiment where low levels of N volatilization (<15% at the highest rates) and invariable nitrate concentration along the soil profile were observed (De Prado et al. 2001). Nonetheless, volatilization may have played a role in the tall wheatgrass experiment during autumn (TW99a), in which high soil temperature and pH lead to the loss of up to 35% of the applied N (Barbieri et al. 2006).

Conclusions

The present study extends to marginal soils, to species adapted to such environment (e.g. tall wheatgrass),

and to winter growth conditions (freezing temperatures) the empirical support for the use of N_{ref} in the quantification of the degree of N limitation to pasture growth. Obtained results encourage the use of the NNI, calculated as the ratio of actual N concentration to N_{ref}, as a valid index to orientate on the requirement of N fertilizer supply when the environmental as well as the pasture conditions are adequate for eventually reaching potential growth.

However, it also shows that the relationship between the critical N concentration and shoot biomass varied depending on growth conditions. Thus, when factors other than N are expected to limit pasture growth (e.g. water stress, deficiencies of another nutrient), an N_{cr} lower than N_{ref} should be used. This is a central point to take into account for diagnose purposes, particularly when economical as well as environmental outcomes are considered. Early diagnosis of N deficiency through the use of initial NNI—corresponding to the ratio between the actual N concentration and the N_{ref} at the initial stages of regrowth (around 150 to 200°C day)—could be a promising tool to predict both the productive capacity of the pasture as well as to estimate N fertilization rates.

References

Agnusdei MG, Colabelli ME, Fernández Grecco RC (2001) Crecimiento estacional de forraje de pasturas y pastizales naturales para el sudeste bonaerense. Boletín Técnico N°152. CERBAS, INTA EEA Balcarce, 16 p

Barbieri PA, Echeverría HE, Sainz Rozas HR, Picone L (2006) Nitrogen use efficiency from urea applied to a tall wheatgrass (*Elytrigia elongata*) praire in a sodic soil. Aust J Exp Agr 46:535–543

Bélanger G, Richards JR (1997) Growth analysis of timothy grown with varying N nutrition. Can J Plant Sci 77:373–380

Bélanger G, Ziadi N (2008) Phosphorus and nitrogen relationships during spring growth of an aging Timothy Sward. Agron J 100:1757–1762

Bélanger G, Gastal F, Lemaire G (1992) Growth analysis of a tall fescue sward fertilized with different rates of nitrogen. Crop Sci 32:1371–1376

Bélanger G, Walsh JR, Richards JE, Milburn PH, Ziadi N (2001) Critical nitrogen curve and nitrogen nutrition index for potato in Eastern Canada. Amer J Pot Res 78:355–364

Bélanger G, Tremblay GF, Mongrain D (2008) Yield and nutritive value of the spring growth of an ageing Thimothy Sward. Can J Plant Sci 88:457–464

Borrajo CI (1998) Generación y expansión de los órganos foliares de agropiro alargado en función del material genético y la disponibilidad de nitrógeno. Thesis M Sc, Universidad Nacional de Mar del Plata

Buol SW, Hole FD, McCracken RJ (1989) Soil genesis and classification, 3rd edn. Iowa University Press, Ames, p 446

de Prado MR, Echeverría HE, Lattanzi FA, San Marín NF (2001) Evolución otoño invernal del nitrógeno mineral en un natracuol típico bajo festucas con diferente hábito de crecimiento Ciencia del Suelo. 19:39–46

Duru M, Ducrocq H (1996) A nitrogen and phosphorus herbage nutrient index as a tool for assessing the effect of N and P supply on the dry matter yield of permanent pastures. Nutr Cycl Agroecosyst 47:59–69

Duru M, Lemaire G, Cruz P (1997) The nitrogen requirements of grasslands. In: Lemaire G (ed) Diagnosis on nitrogen status in crops. Springer, Berlin, p 59

Echeverría HE, García FO (1998) Guía para la fertilización fosfatada de trigo, maíz, girasol y soja. Boletín técnico 149. Estación Experimental Agropecuaria INTA Balcarce, 18 p

Falster DS, Warton DI, Wright IJ (2006) SMATR: Standardised major axis tests and routines, ver 2.0. http://www.bio.mq.edu.au/ecology/SMATR/. Cited 9 Oct 2009

García FO (2001) Balance de fósforo en los suelos de la región pampeana. INPOFOS Cono Sur. Informaciones Agronómicas del Cono Sur 9:1–3

García FO (2003) Introducción. Simposio "El fósforo en la agricultura argentina". INPOFOS Cono Sur, Instituto de la Potasa y el Fósforo, Potash & Phosphate Institute, Canada, pp 2–3

Gastal F, Lemaire G (2002) N uptake and distribution in crops: an agronomical and ecophysiological perspective. J Exp Bot 53:789–799

Gastal F, Bélanger G, Lemaire G (1992) A model of the leaf extension rate of tall fescue in response to nitrogen and temperature. Ann Bot 70:437–442

Gosse G, Varlet-Grancher C, Bonhomme R, Chartier M, Allirand JM, Lemaire G (1986) Production maximale de matière sèche et rayonnement solaire intercepté par un couvert végétal. Agronomie 6:47–56

Greenwood DJ, Neeteson JJ, Draycott A (1986) Quantitative relationships for the dependence of growth rate of arable crops to their nitrogen content, dry weight and aerial environment. Plant Soil 91:281–301

Greenwood DJ, Lemaire G, Gosse G, Cruz P, Draycott A, Neeteson JJ (1990) Decline in percentage N of C3 and C4 crops with increasing plant mass. Ann Bot 66:425–436

Greenwood DJ, Gastal F, Lemaire G, Draycott A, Millard P, Neeteson JJ (1991) Growth rate and %N of field grown crops: theory and experiments. Ann Bot 67:181–190

Instituto Nacional de Tecnología Agropecuaria (2009) Información agrometeorológica, datos de la EEA Balcarce. http://www.inta.gov.ar/balcarce/info/meteor.htm. Cited 9 Oct 2009

Justes E, Mary B, Meynard J-M, Machet J-M, Thelier-Huche L (1994) Determination of a critical nitrogen dilution curve for winter wheat crops. Ann Bot 74:397–407

Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biol Biochem 32:1485–1498

Lattanzi FA (1998) Efecto de la fertilización nitrogenada sobre el crecimiento de festucas de tipo templado y mediterráneo. Thesis M Sc, Universidad Nacional de Mar del Plata

Lemaire G, Agnusdei MG (2000) Leaf tissue turn-over and efficiency of herbage utilization. in: Lemaire G, Hodgson, J, de Moraes A, Nabinger C, Carvalho PC de F (eds) Grassland Ecophysiology and Grazing Ecology. CABI, Oxon, p 265

Lemaire G, Denoix A (1987) Croissance estivale en matière sèche de peuplements de fétuque élevée (*Festuca arundinacea* Schreb.) et de dactyle (*Dactylis glomerata* L.) dans l'ouest de la France. II. Interaction entre les niveaux d'alimentation hydrique et de nutrition azotée. Agronomie 7:381–389

Lemaire G, Gastal F (1997) N uptake and distribution in plant canopies. In: Lemaire G (ed) Diagnosis on nitrogen status in crops. Springer, Berlin, p 3

Lemaire G, Salette J (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

Lemaire G, Jeuffroy M-H, Gastal F (2008) Diagnosis tool for plant and crop N status in vegetative stage: theory and practices for crop N management. Eur J Agron 28:614–624

Marino MA (1996) Efecto de la fertilización nitrogenada sobre el crecimiento inverno primaveral, la composición química y calidad del forraje de *Avena sativa* y *Lolium multiflorum* Lam. Thesis M Sc, Universidad Nacional de Mar del Plata

Marino MA, Berardo A (2000) Fertilización fosfatada de pasturas en el sudeste bonaerense. II—Efecto de la aplicación de nitrógeno sobre la respuesta a fósforo. Rev Arg Prod Anim 20:113–121

Marino MA, Mazzanti A, Assuero SG, Gastal F, Echeverría HE, Andrade F (2004) Nitrogen dilution curves and nitrogen use efficiency during winter-spring growth of annual ryegrass. Agron J 96:601–607

Mazzanti A, Lemaire G, Gastal F (1994) The effect of nitrogen fertilization upon the herbage production of tall fescue swards continuously grazed by sheep. 1. Herbage growth dynamics. Grass Forage Sci 49:111–120

Moscatelli G, Luters JA, Gómez LA (2009) Niveles de disponibilidad y reservas de potasio en Argentina. International Potash Institute. http://www.ipipotash.org/udocs/Sesion%20l.pdf. Cited 9 Oct 2009

Nelson DW, Sommers LE (1973) Determination of total nitrogen in plant material. Agron J 65:109–112

Plénet D, Cruz P (1997) The nitrogen requirement of major agricultural crops: maize and sorghum. In: Lemaire G (ed) Diagnosis on nitrogen status in crops. Springer, Berlin, p 93