



## A species-specific critical nitrogen dilution curve for sunflower (*Helianthus annuus* L.)

P. Debaeke<sup>a,b,\*</sup>, E.J. van Oosterom<sup>c,d</sup>, E. Justes<sup>a,b</sup>, L. Champolivier<sup>e</sup>, A. Merrien<sup>e</sup>, L.A.N. Aguirrezabal<sup>f</sup>, V. González-Dugo<sup>g</sup>, A.M. Massignam<sup>h</sup>, F. Montemurro<sup>i</sup>

<sup>a</sup> INRA, UMR 1248 AGIR, BP 52627, F-31320 Castanet-Tolosan, France

<sup>b</sup> Université de Toulouse, INPT, UMR AGIR, F-31029 Toulouse, France

<sup>c</sup> The University of Queensland, School of Agriculture and Food Sciences, Brisbane, QLD 4072, Australia

<sup>d</sup> The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Brisbane, QLD 4072, Australia

<sup>e</sup> Cetiom, Centre de Grignon, BP 4, 78850 Thiverval-Grignon, France

<sup>f</sup> Unidad Integrada Balcarce, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Instituto Nacional de Tecnología Agropecuaria, CC 276, 7620 Balcarce, Argentina

<sup>g</sup> Instituto de Agricultura Sostenible, CSIC Alameda del Obispo, 14080 Cordoba, Spain

<sup>h</sup> Epagri – Ciram, Rodovia Admar Gonzaga, 1.347, 88034-901 Florianópolis, SC, Brazil

<sup>i</sup> Unità di Ricerca per lo Studio dei Sistemi Culturali, S.S. 106 Jonica km 448.2, 75010 Metaponto, MT, Italy

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### ABSTRACT

For annual and perennial crops, mathematical models have been developed to describe tissue nitrogen (N) dilution during crop growth and to estimate the plant N status applying the N nutrition index (NNI), the ratio between the actual tissue N concentration ([N]) and the tissue N concentration needed to obtain the maximum instantaneous crop growth rate (critical tissue N concentration, [N]<sub>c</sub>). The relationship between shoot [N]<sub>c</sub> and shoot dry matter (DM, t ha<sup>-1</sup>) can be described by an allometric power equation: [N]<sub>c</sub> =  $a \text{ DM}^{-b}$ , where  $a$  and  $b$  are crop-specific parameters. Critical N dilution curves (CND) have been determined for several C<sub>3</sub> crops but not specifically for sunflower (*Helianthus annuus* L.). The objectives of this work were to (i) determine and validate the N dilution curves for critical, minimum, and maximum [N] for sunflower from the juvenile stages to the end of flowering, (ii) compare the critical curve with published CNDs for other C<sub>3</sub> crops, and (iii) estimate the range of variation of NNI for different levels of N fertilization and irrigation. A wide range of field experiments from Argentina, Australia, France, Italy, and Spain was used to establish the dilution curve for sunflower and to independently validate it. The fitted CND [N]<sub>c</sub> =  $4.53 \text{ DM}^{-0.42}$  yielded lower values for [N]<sub>c</sub> than references used until now for diagnosis and decision making in sunflower. The value of parameter  $a$  was generally similar to that of other C<sub>3</sub> species, but the value for parameter  $b$  differed. This was possibly associated with species differences in dry mass partitioning, and justified the development of a sunflower-specific CND. A preliminary reference curve for maximum [N] suggested an evolution from the juvenile stages to the end of flowering similar to that of [N]<sub>c</sub>. Minimum [N], in contrast, appeared to be more constant over time. Relationships between relative grain yield and NNI across a range of locations indicated that in general, maximum grain yield was reached around NNI = 0.8, although at one location this was around NNI = 1.0. The CND can provide useful applications for crop modeling, N status diagnosis, and N fertilization decision.

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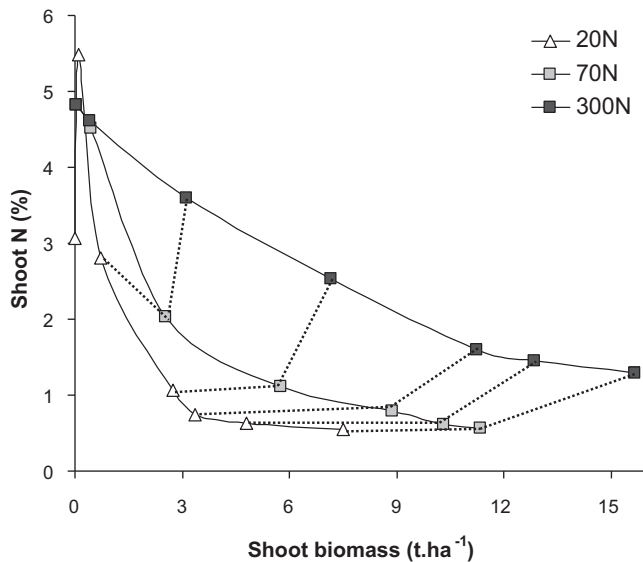
### 1. Introduction

For annual crops, the amount and timing of nitrogen (N) fertilization application represent key decisions in crop management, but only limited published information exists on the response to

nitrogen of sunflower in comparison to cereals such as wheat or maize. Previous studies have shown that N deficiency in sunflower can result in leaf area reduction, poor light interception, and low grain number m<sup>-2</sup> with negative impact on achene yield (Connor and Hall, 1997; Massignam et al., 2009) as it is for most other grain crops. In contrast, excess N in sunflower may result in increased disease damage (Seassau et al., 2010) and reduced oil concentration in achenes (Ozer et al., 2004). Therefore, reliable indicators of crop N status are required to improve detection and correction of N deficiencies, and to evaluate N fertilization strategies in sustainable crop production (Lemaire et al., 2008a).

\* Corresponding author at: INRA, UMR AGIR, BP 52627, Chemin de Borde Rouge Auzeville, 31326 Castanet-Tolosan, France. Tel.: +33 05 61 28 50 16; fax: +33 05 61 73 55 37.

E-mail address: [debaeke@toulouse.inra.fr](mailto:debaeke@toulouse.inra.fr) (P. Debaeke).



**Fig. 1.** Shoot N% versus shoot biomass as a function of N supply across seven sampling dates for exp-8 (Table 1; Massignam et al., 2009). Dotted lines connect data points of samples taken on the same day.

Shoot N concentration ([N]) in crops decreases with increased plant biomass, irrespective of the N nutrition level (Greenwood et al., 1990; Lemaire and Gastal, 1997). An example for sunflower from Massignam et al. (2009) is given in Fig. 1, illustrating the differences in [N] decline as a function of N fertilization rate. The minimum shoot [N] necessary to achieve maximum shoot biomass at any time is defined as the critical shoot [N] ( $[N]_c$ , %) (Lemaire and Salette, 1984; Justes et al., 1994). The relationship between  $[N]_c$  and crop dry matter (DM,  $t\ ha^{-1}$ ) can be described by an allometric power equation, where  $a$  and  $b$  are positive constants (Greenwood et al., 1990):

$$[N]_c = a\ DM^{-b} \quad (1)$$

Parameters  $a$  and  $b$  (Eq. (1)) both have a clear biological meaning in relation to histological, morphological, and ecophysiological characteristics of the different crop species (Lemaire et al., 2008b). Parameter  $a$  represents the shoot [N] at  $DM = 1\ t\ ha^{-1}$ , and thus represents the intrinsic crop N requirements during early growth (Lemaire et al., 2007). Parameter  $b$  describes the pattern of decline in shoot [N] as the crop grows and is thus associated with the ratio between the relative accumulation rates of N and biomass, which in turn is related to dry mass partitioning, e.g. leaf-to-stem ratio (Gastal and Lemaire, 2002). Self-shading of leaves with time is also a process responsible for N dilution as shaded lower leaves contain less N than upper ones (Sadras et al., 1993). In general,  $C_4$  crops have lower values for parameter  $a$  than  $C_3$  crops. This reflects lower N values in  $C_4$  species, associated with lower protein needs for the  $C_4$  metabolism (Greenwood et al., 1990).

The critical N dilution curve (CNDC) defined by Eq. (1) is currently used to diagnose N deficiency, to manage N fertilization, and to simulate N uptake in crop models (Stöckle and Debaeke, 1997; Brisson et al., 2003; Lemaire et al., 2008a). It enables differentiation of three levels of the crop N status: (i) values significantly below the curve represent crop growth limited by N supply, (ii) values above the curve represent growth under luxury N supply, and (iii) values on the curve represent growth at  $[N]_c$ .

Critical dilution curves for [N] have been determined for both  $C_3$  and  $C_4$  crops, including grasses (Lemaire and Salette, 1984; Lemaire and Denois, 1987; Marino et al., 2004), lucerne (Lemaire

et al., 1985), wheat (Justes et al., 1994; Ziadi et al., 2010), maize (Plénet and Lemaire, 1999), sorghum (Van Oosterom et al., 2001), rice (Sheehy et al., 1998), oilseed rape (Colnenne et al., 1998), cotton (Xiaoping et al., 2007), linseed (Flénet et al., 2006), pea (Ney et al., 1997), potato (Bélanger et al., 2001), muskmelon (Fogaca et al., 2008), and tomato (Tei et al., 2002). In a first approximation for  $C_3$  species, Greenwood et al. (1990) proposed a generic critical dilution curve:

$$[N]_c = 5.7\ DM^{-0.50} \quad (2)$$

Although differences among  $C_3$  species are less than those between  $C_3$  and  $C_4$  species, significant differences among  $C_3$  crops for parameters of the CNDC have been reported (Flénet et al., 2006). This may represent differences in underpinning physiological processes, as parameters  $a$  and  $b$  (Eq. (1)) both have biological relevance (Lemaire et al., 2008b).

Sunflower accumulates nitrogen into vegetative plant parts predominantly between the development stages of flower bud and full anthesis (Gachon, 1972). Under optimum conditions, the [N] is about 3-fold to 5-fold greater in leaves than in stems (Merrien, 1992). As the leaf-to-stem biomass ratio steadily decreases with time during the pre-anthesis period (Trapani et al., 1994), shoot [N] decreases as a result of N dilution in stems, despite higher [N] in leaves. This decline in stem [N] is a consequence of an increasing proportion of structural and storage materials that contain little nitrogen (Greenwood et al., 1990). As a consequence, differences in dry mass partitioning can affect N allocation and critical N content. This has been observed in sorghum, where genotypic difference in critical leaf N content could be explained as a consequence of differences in N allocation in response to differences in organ size (Van Oosterom et al., 2010). Hence, species differences in dry mass partitioning could potentially affect the CNDC and justify the development of a sunflower-specific CNDC.

Sunflower is a major  $C_3$  oil crop in the world. Although a number of preliminary dilution curves have been proposed for potential application in sunflower, generally by analogy with other  $C_3$  species (Merrien, 1992; Reau et al., 2001; González-Dugo et al., 2010), a robust sunflower-specific CNDC, based on a comprehensive dataset that includes a wide range of N application rates, has not been determined until now using a statistical approach. Such a sunflower-specific critical curve is necessary for accurate diagnosis of crop N status and hence of fertilization management, and would facilitate applications of crop growth simulation models in crop management. The presence of species differences for CNDC within  $C_3$  crops justifies the development of a species-specific CNDC for sunflower to optimize fertilizer management of sunflower. Hence, the objectives of this study were (i) to determine and validate the dilution curve for the critical, minimum, and maximum shoot [N] of sunflower from early growth stages to flowering, using the method of Justes et al. (1994) across a large international dataset, (ii) to justify the development of sunflower-specific CNDC through comparison with curves developed for other  $C_3$  species, and (iii) to estimate the NNI for different levels of N fertilization and irrigation in order to establish a relationship with grain yield.

## 2. Materials and methods

### 2.1. Experimental details

A wide range of field experiments from Argentina, Australia, France, Italy, and Spain was used to develop (Table 1) and to independently validate (Table 2) the CNDC for sunflower. Data used to develop the dilution curve came from experiments that included three to six N fertilizer rates, ranging from zero to non-limiting

**Table 1**  
Experiments used for establishing the critical N dilution curve for sunflower.

No	Location	Year	Cultivar(s)	N treatments (kg N ha <sup>-1</sup> )	Grain yield range (t ha <sup>-1</sup> )	Sampling dates	References
1	Auzeville (France)	2005	Heliadol – Melody	0–40–80–120–160	3.5–4.3	7	Debaeke and Raffaillac (2006)
2	Surgères (France)	1995	Sankara	0–20–40–60–80–100–120	n.a. <sup>a</sup>	5	Merrien (unpublished)
3	Satolas (France)	1995	Albena	0–30–60–90–120	n.a.	3	Merrien (unpublished)
4	Dijon (France)	1995	Albena	0–30–60–90–120	n.a.	4	Merrien (unpublished)
5	Saint Pathus (France)	1995	Albena	0–30–60–90–120	n.a.	5	Merrien (unpublished)
6	Surgères (France)	2006	Melody	0–50–100–200	2.9–3.2	3	Champolivier (unpublished)
7	Gatton (Australia)	1999	Hysun 36	0–50–250	2.5–3.1	8	Massignam et al. (2009)
8	Gatton (Australia)	1999	Hysun 47	20–70–300	1.1–5.6	9	Massignam et al. (2009)
9	Gatton (Australia)	2001	Hysun 47	0–150–250	4.2–4.6	7	Massignam et al. (2009)
10	Foggia (Italy)	1996–1999	Akiles-Isoleic – Sanbro-Select	0–50–100	2.5–3.9	3	Montemurro and De Giorgio (2005) and De Giorgio et al. (2007)
11	Foggia (Italy)	2001, 2003	Heliogen	0–50–100	n.a.	4	Montemurro et al. (2005)

<sup>a</sup> Data not available.

amounts of N. The number of sampling dates ranged from three to nine and sampling occurred between the B4 (4-leaves) and F4 (late flowering) stages (Cetiom scale, see Merrien, 1992). Because reproductive organs (heads and achenes) of sunflower have contrasting [N] compared to vegetative organs (Merrien, 1992), the presence of a significant proportion of biomass in reproductive organs could affect the CNDC (Lemaire and Gastal, 1997). Therefore, the analyses included only data obtained during the pre-anthesis and flowering period, before significant grain filling occurred. Shoot biomass at early flowering in general did not exceed 9 t ha<sup>-1</sup>. Samples with biomass less than 0.75 t ha<sup>-1</sup> were excluded from the analysis, because of (i) the difficulty to obtain significant plant response to N fertilization during the early vegetative growth stages of sunflower and (ii) the fact that the allometric relationship is quite different during very early growth stages (Justes et al., 1997; Lemaire and Gastal, 1997).

Experimental treatments included a wide range of sunflower cultivars differing in earliness and crop architecture, but only experiments with dense plant population (5.5–8 plants m<sup>-2</sup>) were used in the analyses. Some treatments were irrigated before anthesis, which may have enhanced N uptake. However, most of the treatments were not irrigated and some experienced moderate drought stress at flowering. In general, experiments contained 3–4 replications. N fertilization was applied mainly at sowing, and sometimes also as additional amounts between sowing and flower bud stage (later if fertigation was used). It was applied in mineral (ammonium nitrate, urea) or organic (manure, compost, urban waste) forms.

## 2.2. Biomass and N concentration measurements

Shoot biomass (t ha<sup>-1</sup>) was determined either by randomly cutting 4–6 plants at ground level in different rows, or by sampling an area of 1 m<sup>2</sup> in each plot. Dry matter was determined by oven-drying the samples for at least 2 days at 80 °C before weighing. Samples were subsequently ground to a powder for analytical N determination. Shoot [N] was determined using either the Kjeldahl 'classic' method or the Dumas method for the more recent data. Even though only the latter method takes into account the nitrate in the plant, this represents only a small fraction of the total amount of nitrogen present in the crop when N fertilization is not abundant (Justes et al., 1994).

## 2.3. Construction and validation of the critical, minimum, and maximum N dilution curves

The classical approach to determine the CNDC (Justes et al., 1994) was applied, with biomass samples used initially restricted to plant stands > 5.5 plants m<sup>-2</sup> and DM > 0.75 t ha<sup>-1</sup>. For each harvest date in each experiment, shoot DM produced and shoot [N] were compared across the different N treatments by analysis of variance using Statistix 9.0 (Tallahassee, FL, USA). Homogeneous groups of means were determined by LSD method at  $P < 0.10$ . This significance level was preferred over the classically used  $P < 0.05$  in order to reduce the occurrence of Type II errors that could be high in such field experiments.

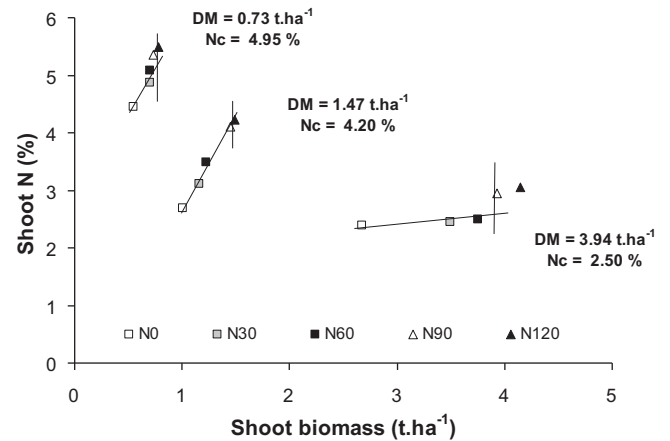
For each measurement date, the variation in shoot [N] versus shoot DM across the 3–5 different N levels was combined into a bilinear relation composed of (a) a linear regression representing the joint increase in [N] and DM and (b) a vertical line corresponding to an increase in [N] without significant variation in shoot DM. Maximum shoot biomass was calculated as the average of the observed data in non-limiting N conditions. The theoretical [N]<sub>c</sub> corresponds to the ordinate of the breakpoint of the bilinear regression. An example of the method used for the determination of DM and [N]<sub>c</sub> is given in Fig. 2, where the response of shoot biomass to five N fertilization rates was compared at three growth stages (exp-3, Table 1). Consistent with previous studies, a power regression (Freundlich model) was used to fit the observed decrease in [N]<sub>c</sub> with increasing shoot biomass.

Developing CNDC requires a very large number of experiments with multiple sampling dates. A total of 204 experimental series from 25 datasets where [N] and shoot biomass were measured at a given growth stage for at least 3 contrasting N rates and 3–4 replicates were potential candidates for CNDC establishment (Tables 1 and 2). After statistical tests, only 45 of these (22%) that allowed determination of maximum shoot biomass unambiguously were retained (Table 1). The 11 experiments in Table 1 that were used to establish the critical curve were either irrigated or experienced no severe water deficit until anthesis to avoid potential confounding effects of drought stress on the CNDC.

The CNDC was validated on data from experiments independent from those used for its development (Table 2). These data sets were also used to determine the [N]<sub>min</sub> and [N]<sub>max</sub> curves. From the experiments in Table 2, sampling dates were selected for which N supply significantly affected biomass accumulation.

**Table 2**  
Experiments used for validating the critical N dilution curve for sunflower.

No	Location	Year	Cultivar(s)	N treatments (kg N ha <sup>-1</sup> )	Grain yield range (t ha <sup>-1</sup> )	Sampling dates	References
12	Auzeville (France)	1987	Pharaon	0–70–140–210–260	n.a.	3	Blanchet (unpublished)
13	Auzeville (France)	1989	Pharaon – TN15	0–90–270	1.6–4.1	5	Blanchet (unpublished)
14	Auzeville (France)	1990	Pharaon – TN15	0–90	1.8–4.2	3	Blanchet (unpublished)
15	Auzeville (France)	1995–2002	Albena–Asturia–Cristo– Labrador–Melody–Santiago	60–120	1.9–4.2	2	Debaeke et al. (2003)
16	Auzeville (France)	2004–2006	Melody	0–60	n.a.	1	Justes (unpublished)
17	Auzeville (France)	2002	Albena–Heliasol–LG5660 Melody–Primasol–Prodisol Santiago–Vidoc	25–70–105	2.3–3.9	3	Debaeke (unpublished)
18	Auzeville (France)	2003	AlStar	75	3.5	2	Debaeke (unpublished)
19	Auzeville (France)	2004	Prodisol	40–80–120	3.4–4.4	3	Debaeke (unpublished)
20	Auzeville (France)	2006	Heliasol–Melody	0–60–100–160	2.6–4.2	4	Debaeke (unpublished)
21	Auzeville (France)	2007–2009	Heliasol	0–50–75–150	2.0–4.4	1	Debaeke (unpublished)
22	France: 18 trials	1992–2002	Albena–AlStar–Euroflor– Frankasol–Heliasol– Melody–Prodisol–Santiago– Viki–Voltoal	0–20–40–60–80–100–120–140	1.5–4.0	3	CETIOM (unpublished)
23	France: 41 trials	2000–2001	–	0–50	2.4–4.2	3	CETIOM (unpublished)
24	Cordoba (Spain)	1985	Sungro380	0–75–210	1.6–5.4	3	Alvarez de Toro (1987)
25	Balcarce (Argentina)	1997	Contiflor 9	0–60–120	1.5–2.6	4	Redolatti et al. (2000)

**Fig. 2.** An example of the method used to determine maximal shoot biomass (DM) and critical N concentration ( $[N]_c$ ) on a field experiment comparing crop growth as a function of 5 N fertilization rates (0, 30, 60, 90 and 120 kg N ha<sup>-1</sup>) applied at sowing for exp-3 (Table 1).

A treatment was considered to be N limiting when its shoot DM was significantly smaller ( $P < 0.10$ ) than the shoot DM of the treatment having received the rate of fertilizer-N immediately higher. In most cases, N-limiting treatments included those with N rates  $< 40$  kg ha<sup>-1</sup> (generally 0 N check plots) and, in drought-prone environments, non-irrigated treatments where N uptake was obviously reduced. On the other hand, only irrigated treatments that received more than 100 kg N ha<sup>-1</sup> were assumed to be non-limiting, because sunflower crops require about 180–200 kg ha<sup>-1</sup> for maximizing grain yield, with residual N from the previous crop and soil mineralization contributing approximately two-thirds of this requirement (Merrien, 1992).

The lower boundary of  $[N]$  fitted from the most N limiting experiments was assumed to represent the  $[N]_{\min}$  curve. Although drought during vegetative stages can induce N stress in sunflower, direct (via N supply) and indirect (via drought) N stress both result in low  $[N]$ , and hence could both be used for the determination of the  $[N]_{\min}$  curve. The upper boundary of  $[N]$  fitted from the most plethoric N treatments was assumed to represent the  $[N]_{\max}$  curve. Situations where obvious soil water deficit had occurred before flowering were discarded from this evaluation because even high N fertilization rates could result in reduced N uptake in such conditions.

#### 2.4. Calculation of nitrogen nutrition index (NNI)

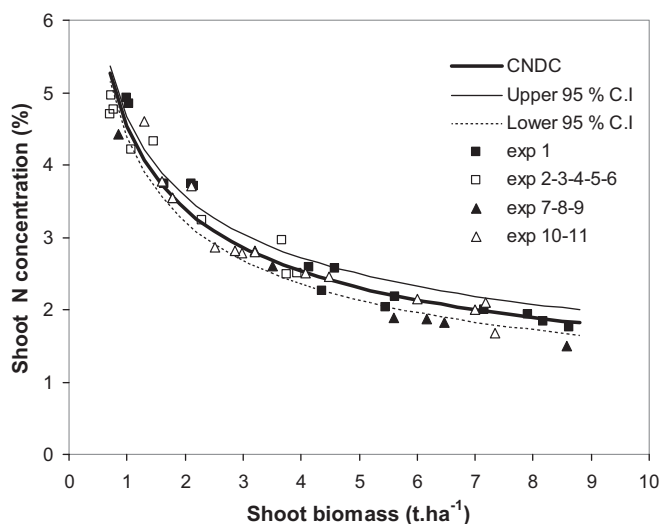
The CNDC should allow an accurate diagnosis of sunflower N nutrition status up to anthesis. To characterize the N status of plants, the NNI (nitrogen nutrition index) was calculated as follows (Lemaire and Gastal, 1997):

$$NNI = \frac{[N]_a}{[N]_c} \quad (3)$$

where  $[N]_a$  is the actual and  $[N]_c$  the critical N concentration in shoot dry matter prior to anthesis. If  $NNI = 1$ , N nutrition is considered optimal, while  $NNI > 1$  indicates excess N and  $NNI < 1$  indicates N deficiency. Therefore, NNI can be used for quantifying the degree of N stress after the onset of N deficiency.

A subset of experiments from Tables 1 and 2 where grain yield was determined together with biomass and  $[N]$  around anthesis for at least three levels of N fertilization was used to establish the relationship between relative grain yield and NNI. Relative yield was determined as the ratio of yield to maximum yield in each experiment and for each cultivar.





**Fig. 3.** Critical N concentration versus shoot biomass for sunflower data across a range of experiments (see Table 1). Fitted function represents the critical N dilution curve (CNDC) for biomass data in the range 0.7–8.6 t DM ha<sup>-1</sup>:  $[N]_c = 4.53 \text{ DM}^{-0.42}$ . Thin lines represent the upper and lower 95% confidence intervals.

### 3. Results

#### 3.1. Establishment of the critical N dilution curve

A set of 45 sampling dates from 11 experiments (Table 1), which included shoot DM values between 0.75 and 8.60 t ha<sup>-1</sup>, was used to calculate  $[N]_c$  for a given level of biomass (Fig. 3). Only the sampling dates satisfying the statistical requirements described in Section 2.3 were kept for the establishment of CNDC. These data corresponded to growth stages ranging from E1 (visible flower bud) to mid flowering (F2) and thus excluded the grain filling period. A power function was fitted to the calculated  $[N]_c$  (Fig. 3):

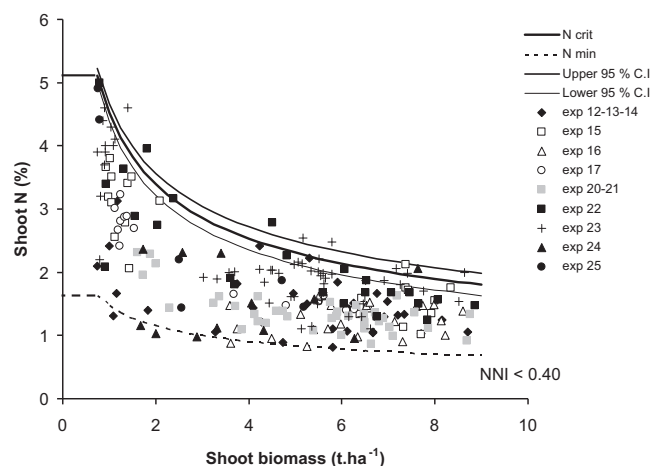
$$[N]_c = 4.53 \text{ DM}^{-0.42} \quad (4)$$

with 95% confidence intervals of [4.39; 4.67] for  $a$  and [0.39; 0.45] for  $b$ . In the biomass range 0.1–0.75 t ha<sup>-1</sup>, which corresponds to growth stages prior to flower bud appearance, increasing N rates at sowing did not significantly affect shoot biomass, because N requirement is relatively low during these early growth stages. Consequently, the CNDC could not be applied easily at early growth stages. The  $[N]_c$  value corresponding to DM of 0.75 t ha<sup>-1</sup> would be 5.1% based on extrapolation of Eq. (4). Consistent with this, the average  $[N]$  for the 95 samples with shoot biomass less than 0.75 t ha<sup>-1</sup> was 5.08%. For that reason, and in accordance with the method used for wheat (Justes et al., 1994),  $[N]_c$  was set to 5.1% for shoot biomass of sunflower less than 0.75 t ha<sup>-1</sup>.

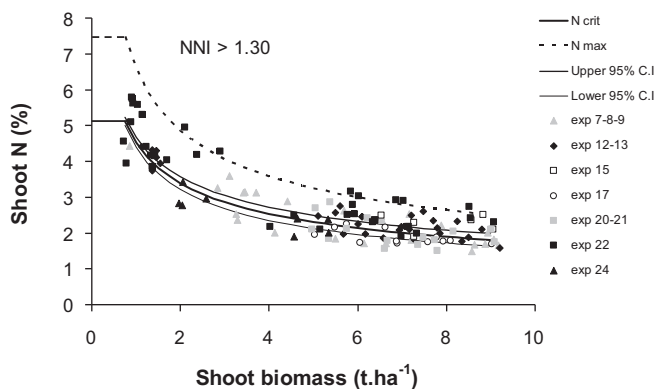
#### 3.2. Validation of the critical N dilution curve

The critical curve was validated both for limiting (Fig. 4) and non-limiting (Fig. 5) situations within the biomass range for which it was established. Results indicated that growth rate, crop density (between 3.5 and 6.9 plants m<sup>-2</sup>), and cultivar (24) did not significantly affect  $[N]_c$ . The wide range of pedoclimatic conditions included in our dataset (Western and Southern Europe, Argentina, and Australia) suggests that the sunflower CNDC was also independent of the growing environments.

In general, values for shoot  $[N]$  from N-limiting treatments were close to or lower than the CNDC (Fig. 4), whereas those from experiments where N was expected to be non-limiting were close to



**Fig. 4.** Shoot N concentration versus shoot biomass (DM) for sunflower data in treatments that experienced N stress ( $n = 240$ ). Solid lines represent the critical N dilution curve plus 95% confidence intervals. Dotted line represents the minimum N concentration ( $[N]_{\min}$ ).  $[N]_{\min} = 1.47 \text{ DM}^{-0.34}$  corresponding to  $\text{NNI} = 0.40$ .



**Fig. 5.** Shoot N concentration versus shoot biomass (DM) for sunflower data in treatments without N stress ( $n = 136$ ). Solid lines represent the critical N dilution curve plus 95% confidence intervals. Dotted line represents the maximum N concentration ( $[N]_{\max}$ ).  $[N]_{\max} = 6.49 \text{ DM}^{-0.44}$  corresponding to  $\text{NNI} = 1.30$ .

or above the CNDC (Fig. 5). To determine the  $[N]_{\min}$ , only observations for which NNI was less than 0.4 were considered ( $n = 11$ ) and for  $[N]_{\max}$  curve, only observations for which NNI exceeded 1.3 were selected ( $n = 12$ ). From the available data, boundary curves for  $[N]_{\min}$  and  $[N]_{\max}$  were calculated for the 0.75–9 t ha<sup>-1</sup> DM range without imposing fixed values to parameters  $a$  and  $b$ :

$$[N]_{\min} = 1.47 \text{ DM}^{-0.34} \text{ for } \text{DM} \geq 0.75 \text{ t ha}^{-1} \quad (5)$$

$$[N]_{\min} = 1.63 \text{ for } \text{DM} < 0.75 \text{ t ha}^{-1}$$

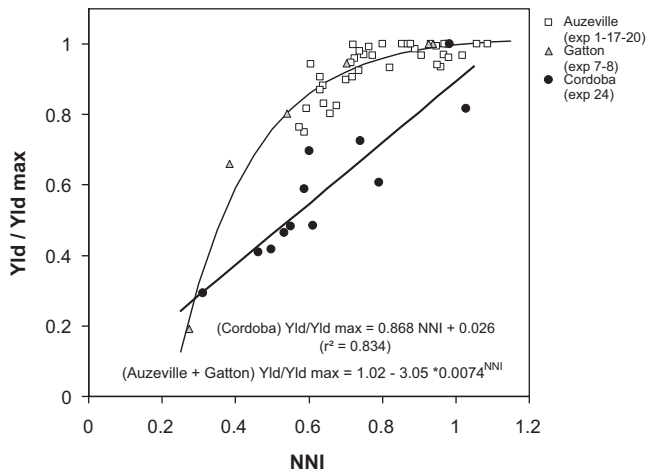
$$[N]_{\max} = 6.49 \text{ DM}^{-0.44} \text{ for } \text{DM} \geq 0.75 \text{ t ha}^{-1} \quad (6)$$

$$[N]_{\max} = 7.48 \text{ for } \text{DM} < 0.75 \text{ t ha}^{-1}$$

The confidence intervals of the  $b$  parameter were [0.22; 0.47] for  $[N]_{\min}$  and [0.37; 0.50] for  $[N]_{\max}$ . Both values for  $b$  were therefore not significantly different from the value for  $[N]_c$ .

#### 3.3. Relationship between relative grain yield and NNI

Relative grain yield (yield/yield max) was plotted against NNI at anthesis for seven experiments across three locations (Auzeville, Cordoba, Gattón) from Tables 1 and 2 where at least (i) three N fertilization rates were compared and (ii) one N level resulted in NNI value greater or equal to 1 (Fig. 6). In all three locations, both NNI



**Fig. 6.** Statistical relationships between relative grain yield (yield/yield max) and nitrogen nutrition index (NNI) for selected experiments from Tables 1 and 2.

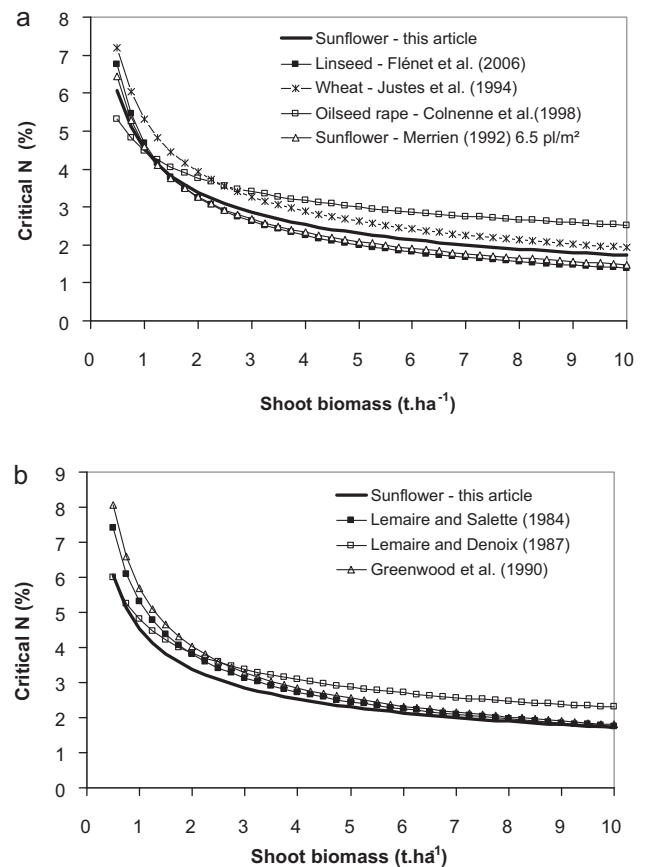
and relative yield increased with N fertilization levels, until a critical level was reached at which yield did not increase anymore with increasing NNI. In Cordoba (Spain), grain yield increased linearly with NNI over the range of N fertilization and supplementary irrigation, such that maximum yield was reached around NNI = 1.0. In the other locations, however, the relationship between grain yield and NNI was non-linear, as maximum grain yield was reached at lower NNI. At NNI = 0.8, yield as a percentage of maximum yield was around 96% in Auzeville (France) and Gatton (Australia).

#### 4. Discussion

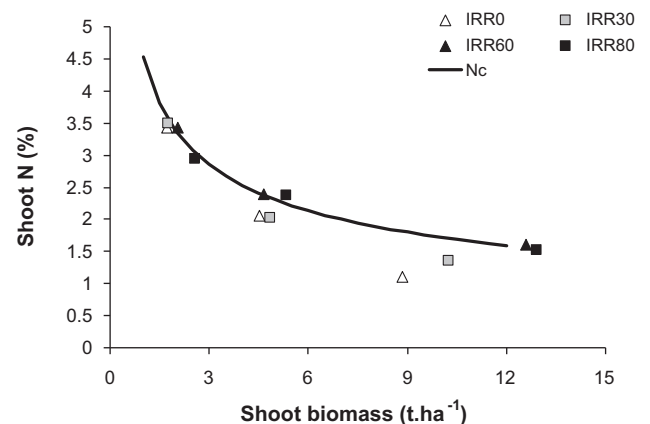
The results presented in this paper validate for sunflower the concept of N dilution with shoot biomass, which was first developed for herbaceous annual species by Greenwood et al. (1990), then extended to a range of C<sub>3</sub> and C<sub>4</sub> crops. The concept considers that the shoot [N] decreases with increasing plant biomass (allometric relationship) and that a dilution curve for [N]<sub>c</sub> can be described by a negative power function.

##### 4.1. Species specificity of sunflower CNDC is likely associated with dry mass partitioning

The comprehensive dataset used in this analysis resulted in a sunflower-specific CNDC that was close to the preliminary CNDC reported by Merrien (1992), which was based on a limited dataset (Table 3 and Fig. 7a). Species differences in the response of [N]<sub>c</sub> to increasing DM have been reported in the literature (Table 3 and Fig. 7). Except at low shoot biomass, sunflower CNDC was considerably lower than the values for oilseed rape (Colnenne et al., 1998) and winter wheat (Justes et al., 1994) but greater than that of linseed (Flénet et al., 2006) (Fig. 7a). Estimates for sunflower for both parameters *a* and *b* were in the middle of the range of reported values for C<sub>3</sub> crops. Nonetheless, many crops had values outside the confidence interval observed for sunflower, particularly for parameter *b* (Table 3). However, because parameter values are determined statistically, it is possible that any species differences in parameter values are a statistical artifact with little biological meaning (Gastal and Lemaire, 2002). Errors in the estimates of parameters *a* and *b* could indeed account for the relatively large number of shoot [N] data located below the CNDC for treatments where N supply was not limiting (Fig. 5). There are, however, also other possible reasons for these errors in Fig. 5. Firstly, although treatments with severe drought stress were excluded from the analyses, it is possible that in some cases moderate drought stress



**Fig. 7.** Comparison of the sunflower N critical dilution curve with other C<sub>3</sub> crops for (a) cereals and oil crops and (b) grasses and a generic C<sub>3</sub> curve used for diagnosis of crop N status.



**Fig. 8.** Relationship between shoot N concentration (%) and shoot biomass (t.ha<sup>-1</sup>) for 4 irrigation levels (0, 30, 60 and 80% of full irrigation of 465 mm) under the highest N fertilization level (210 kg N.ha<sup>-1</sup>) of exp-24 (Table 2), Cordoba (Spain), 1985. Bold line is the critical N dilution curve determined in Fig. 3. Based on tabulated data from Alvarez de Toro (1987).

may have occurred toward anthesis only, which may have limited N uptake more than plant growth. This is illustrated in Fig. 8 for data from Cordoba 1985 (exp-24, Table 2), where only the two highest levels of irrigation were acceptable for the establishment of CNDC. Secondly, differences between the Dumas and Kjeldahl analytical methods could have contributed to errors. Accumulation of nitrate, which is not captured by the Kjeldahl method, is most likely to occur when N is not limiting. Although this error is unlikely to be

**Table 3**Comparison of the parameters of the critical N dilution curve among C<sub>3</sub> species. Species have been sorted for increasing value for parameter *a*.

Crop	<i>a</i>	<i>b</i>	References
Spring wheat ( <i>Triticum aestivum</i> L.)	3.85	0.57	Ziadi et al. (2010), Canada
Annual ryegrass ( <i>Lolium multiflorum</i> Lam)	4.07	0.38	Marino et al. (2004), Argentina
Cotton ( <i>Gossypium hirsutum</i> L.)	4.30/4.97 <sup>a</sup>	0.13	Xiaoping et al. (2007), China
Oilseed rape ( <i>Brassica napus</i> L.)	4.48	0.25	Colnenne et al. (1998), France
Tomato ( <i>Lycopersicon esculentum</i> Mill.)	4.53	0.33	Tei et al. (2002), Italy
Sunflower ( <i>Helianthus annuus</i> L.)	4.53	0.42	This article (Fig. 3)
	4.59 <sup>2</sup>	0.49 <sup>b</sup>	Merrien (1992)
Potato ( <i>Solanum tuberosum</i> L.)	4.57/5.04 <sup>a</sup>	0.42	Bélanger et al. (2001), Canada
Linseed ( <i>Linum usitatissimum</i> L.)	4.69	0.53	Flénet et al. (2006), France
Tall Fescue ( <i>Festuca arundinacea</i> Schreb.)	4.80	0.32	Lemaire and Denois (1987), France
Cocksfoot ( <i>Dactylis glomerata</i> L.)			
Pea ( <i>Pisum sativum</i> L.)	5.10	0.32	Ney et al. (1997), France
Muskmelon ( <i>Cucumis melo</i> L.)	5.16	0.63	Fogaca et al. (2008), Brazil
Grasslands (gramineous)	5.31	0.48	Lemaire and Salette (1984), France
Winter wheat ( <i>Triticum aestivum</i> L.)	5.35	0.44	Justes et al. (1994), France
C <sub>3</sub> (generic)	5.70	0.50	Greenwood et al. (1990)

<sup>a</sup> Varietal effect.<sup>b</sup> Merrien (1992) suggested a preliminary CNDC from a limited number of fertilization experiments, in which DM was expressed per plant (DMp, g plant<sup>-1</sup>): [N]<sub>c</sub> = 281.5/(DMp + 52.6). When converted into a power function and assuming a density of 6.5 plants m<sup>-2</sup>, it becomes: [N]<sub>c</sub> = 4.59 DM<sup>-0.49</sup>. In this conversion, coefficient *a* increases from 4.23 to 4.92 if plant density increases from 5.5 to 7.5 plants m<sup>-2</sup>.

large at [N]<sub>c</sub>, the risk of underestimating [N] is more likely under high N conditions. Therefore, in order to judge the relevance of species differences in parameters of the CNDC, it is important to not just look at statistical differences, but consider the biological implications of different parameter values as well.

Mathematically, parameter *a* represents the shoot [N] when biomass is 1 t ha<sup>-1</sup>. Early in the season, until biomass reached 0.75 t ha<sup>-1</sup>, [N]<sub>c</sub> of sunflower was assumed to be 5.1%, independent of shoot biomass (Figs. 4 and 5). This constant value was justified, because during early stages of plant development, plants grow predominantly in isolation with limited competition for light. Under such circumstances, decline in [N] with biomass accumulation is limited (Lemaire and Gastal, 1997). In addition, shoot biomass during this period consists predominantly of leaf mass, and allocation of biomass to leaves is constant prior to stem elongation (Massignam, 2003). Indeed, the end of the phase of constant CNDC coincided with the onset of stem elongation, when around 10–12 leaves had expanded (Massignam, 2003). The value for parameter *a* (4.53, Eq. (4)) was slightly lower than the [N]<sub>c</sub> of 5.1%, because biomass of 1 t ha<sup>-1</sup> occurred slightly beyond the period of constant [N]<sub>c</sub>, when [N]<sub>c</sub> had already started to decline. In contrast, for C<sub>3</sub> cereals, including winter wheat, Justes et al. (1994) reported a value for parameter *a* of 5.35 (Table 3), that exceeded the [N]<sub>c</sub> of 4.4% during early growth, likely because the period of constant [N]<sub>c</sub> continued until biomass was 1.5 t ha<sup>-1</sup>. For oilseed rape, Colnenne et al. (1998) reported an [N]<sub>c</sub> during early growth of 4.6%, close to the value for parameter *a* of 4.48 (Table 3). Small differences in the value for parameter *a* among C<sub>3</sub> species (Table 3) thus do not necessarily reflect differences in early season shoot [N]. Because the value for parameter *a* for sunflower was in the middle of the range of other C<sub>3</sub> crops (Table 3) and because the main species differences for this parameter are between C<sub>3</sub> and C<sub>4</sub> (Lemaire et al., 2007), we conclude, consistent with results for linseed (Flénet et al., 2006), that any differences in the CNDC of sunflower compared with other C<sub>3</sub> crops are unlikely to be predominantly associated with early season [N] (parameter *a*) and therefore with intrinsic N uptake.

Parameter *b* describes the decline in shoot [N] with crop growth and therefore depends on N uptake relative to biomass accumulation. The decline in shoot [N] during vegetative growth can be attributed to an increase in the proportion of structural and non-photosynthetic biomass with low [N], and a decline in the proportion of metabolic biomass with high [N] (Caloin and Yu, 1984). As stems tend to have a higher proportion of structural biomass

than leaves, shoot [N] tends to decline during stem elongation. For example, for sunflower crops grown under optimum conditions, the contribution of leaves to total biomass during the pre-anthesis period declines from around 0.42 to 0.15, whereas the contribution of stems increased from 0.36 to 0.60 (Trapani et al., 1994). Species or genotypic differences in biomass partitioning could therefore result in different values for parameter *b*. Because such differences in biomass partitioning are not necessarily related to early-season shoot [N], when most biomass is metabolic, values for parameters *a* and *b* across C<sub>3</sub> species (Table 3) were not correlated (*R*<sup>2</sup> = 0.02). Hence, differences in observed values for parameter *b* are unlikely to be a consequence of differences in values for parameter *a*.

For sunflower, the value of parameter *b* is in the middle of the range of published values for C<sub>3</sub> species (Table 3), although most of these fell outside the 95% confidence interval for sunflower (0.39–0.45). Most notably, musk melon, spring wheat, and linseed had greater values, whereas oilseed rape and cotton had lower values. For oilseed rape, dry mass allocation to the stem is low for most of the pre-anthesis period (Gabrielle et al., 1998), what is consistent with the low value for parameter *b* (Table 3). Similarly, the low value for parameter *b* for cotton could be associated with relatively low biomass partitioning to the stem. For cotton, stem dry mass at the onset of boll growth is only marginally greater than leaf mass (Yates et al., 2010), whereas for sunflower, stem mass is around 50% greater than leaf mass at the onset of biomass accumulation in the head (Trapani et al., 1994). Such species differences in dry mass partitioning thus justify a species-specific CNDC for sunflower. However, genotypic differences in pre-anthesis partitioning coefficients to plant parts appear to be relatively small in sunflower (Trapani et al., 1994) and no differential light-associated N profiles have been observed in canopies of sunflower before anthesis (Sadras et al., 1993), even though genotypic differences in leaf area development of sunflower have been reported (Pereyra-Irujo et al., 2008). Therefore, a species-specific sunflower CNDC appears to be adequate for diagnostic and decision making applications of current cultivars.

The boundary curve for [N]<sub>max</sub> had a value for parameter *b* that was not significantly different from that for the CNDC, indicating that biomass partitioning remains relatively constant when N uptake exceeds the CNDC. This is consistent with the concept of [N]<sub>c</sub>, which represents the lowest [N] at which maximum biomass accumulation occurs. This implies that under conditions of luxury N, when [N] exceeds CNDC, biomass accumulation does not

increase with [N] and hence, dry mass partitioning will be similar, resulting in similar value for parameter  $b$ . In contrast, for the  $[N]_{\min}$  curve under N stress, the value for parameter  $b$  tended to be slightly lower than the CNDC curve. As the effect of N stress on biomass partitioning in sunflower is relatively small (Massignam et al., 2009), it is unlikely this relatively low value for  $b$  was associated with a change in partitioning. Rather, it is possible that  $[N]_{\min}$  early in the season was overestimated, as even soil N mineralization can be sufficient to meet crop N demand during very early growth stages. Within this context, Plénet and Lemaire (1999) suggested for maize that the minimum shoot [N] was constant throughout the crop cycle, and they suggested a value of 0.7% for maize. A closer look at observed  $[N]_{\min}$  indicates that beyond a shoot biomass of  $2 \text{ t ha}^{-1}$ , minimum shoot [N] was independent of biomass, and the average of the observed values was 0.9%. Therefore, we suggest that for maximum shoot [N] a dilution curve with a similar value for parameter  $b$  is used as the CNDC, whereas for minimum shoot [N] a constant value of 0.9% is used.

#### 4.2. Application of dilution curves in sunflower crop management and modeling

A main application of CNDC is to diagnose plant N status in agronomic trials and farm surveys. For that purpose, optimal values of NNI should be determined at main growth stages to guide crop management in sunflower. Our analyses (Fig. 6) indicated that grain yield often does not increase much and may sometimes even decrease if NNI exceeds 0.8. This could be due to increased disease damage or potential drought stress occurrence under well-fertilized conditions in sunflower. For instance, Seassau et al. (2010) observed a regular increase of phoma black stem (*Phoma macdonaldii*) incidence and severity in sunflower with increasing NNI. In rainfed potato, Bélanger et al. (2001) observed a decrease in relative yield if NNI exceeded, although relative yield remained near 1 with irrigation. However, in environments where water stress, lodging, and diseases can be prevented through management or genetics, targeting an NNI value of 1 could be valuable for agronomic reasons, although the economic and environmental benefits are less certain. In addition, oil concentration in achenes markedly decreases if NNI exceeds 1 (Debaeke and Raffaillac, 2006). In contrast to cereals, for which in general an NNI of 1 should be targeted in order to maximize yield and grain protein concentration (Debaeke et al., 2006), results for sunflower indicate that NNI levels of 0.8–0.9 at anthesis appear sufficient to maximize both grain yield and oil concentration.

The direct use of CNDC for N fertilization decisions is often limited in sunflower, as most of the N is applied at sowing. However, as management options include either a delayed or a split N application, a fertilization decision support method for sunflower, based on CNDC (Heliotest), was developed and tested in France (Reau et al., 2004). This method used the equation proposed by Lemaire and Salette (1984) for  $C_3$  grasses,  $[N]_c = 5.31 \text{ DM}^{-0.48}$ , with DM ranges from 1 to  $12 \text{ t ha}^{-1}$ , to describe the N nutrition status for sunflower. For the same purpose, González-Dugo et al. (2010) used  $[N]_c = 4.80 \text{ DM}^{-0.32}$ , based on tall fescue and cocksfoot for DM ranges from 1 to  $6 \text{ t ha}^{-1}$  (Lemaire and Denoix, 1987). As illustrated in Fig. 7b, using generic or  $C_3$  grass values would overestimate  $[N]_c$  for a given shoot biomass. Using decision methods based on such reference curves would overestimate the importance of N deficiency and could result in excess N fertilization (e.g. Reau et al., 2001). In contrast, the sunflower-specific equation proposed by Merrien (1992), which has been extensively used in South America to diagnose the nutritional status of sunflower crops (Sosa et al., 1999; de Caram et al., 2007), would slightly underestimate the importance of N deficiency. Using a CNDC reference developed for another  $C_3$  crop could result in too little or too much N fertilizer

being applied, resulting in either N deficiency and yield loss and hence income loss, or over-fertilization, where extra N has no yield benefit and income is lost as well.

Although NNI is a relevant measure of nutrition status of the crop, its determination is time consuming, as it requires successive biomass sampling for determination of DM content, and laboratory analysis of plant N concentration. Hence, NNI is preferably considered as a reference indicator of plant N status, rather than an operational method for assessing the N status of a given crop. Using a preliminary CNDC, Debaeke and Raffaillac (2006) observed a good correlation between NNI and normalized SPAD values as indicated by a chlorophyll meter (SPAD-502, Konica, Minolta, Osaka, Japan). This indirect method could potentially be an alternative for estimating NNI and characterize crops and environments in situations where NNI cannot be measured directly, as was previously proposed for small-grain cereals (Debaeke et al., 2006).

The relationships proposed for  $[N]_c$ ,  $[N]_{\min}$ , and  $[N]_{\max}$  can also be used in the parameterization and validation of crop growth models for predicting the N response and/or N requirement of sunflower. For instance, the SUNFLO model (Casadebaig et al., 2011) uses dilution curves for  $[N]_c$  and  $[N]_{\max}$  to determine N stress occurrence and the maximal capacity of N accumulation in plant tissues, respectively. Other generic models applicable to sunflower, such as STICS (Brisson et al., 2003), could benefit from the parameters developed in this study on a large data set to increase the domain of the genotype  $\times$  environment  $\times$  management landscape in which their N diagnosis methods are valid.

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