

# Diagnosis tool for plant and crop N status in vegetative stage Theory and practices for crop N management

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## Abstract

The environmental constraints to agriculture imply that nitrogen (N) fertilizer management should be adjusted to crop N requirements determined by target yields. Nowadays for environmental and economical reasons target yield of farmers can be lower than the potential crop yields as permitted by soil and climatic conditions. So it is important to provide farmers crop N status diagnostic tools in order to decide the rate and the timing of N fertilizer applications. Theory on crop N uptake and allocation allows the determination of a diagnostic tool, the Nitrogen Nutrition Index, based on the determination of the critical N dilution curve for each crop species considered. During the vegetative growth period of all the crop species studied, including C3 and C4 species and monocots and dicots, plant N concentration decreases monotonically as crop grows because of (i) the ontogenetic decline in leaf area per unit of plant mass, and (ii) the remobilisation of N from shaded leaves at the bottom of the canopy to well illuminated leaves at the top. NNI appears then as an indicator well connected with the physiological regulation of N uptake at canopy level. So this indicator can be used as the basis for determination of crop N nutrition status, and then for decision making on the necessity of an N application for achieving target yield. Nevertheless despite its high physiological relevance, NNI cannot be used directly in farm conditions because its determination is very time consuming. So it is necessary to develop indirect methods for NNI estimation through more operational procedures. Several methods have been proposed in literature, such as nitrate concentration in sap or chlorophyll meter. But the calibration or validation of these methods with NNI have not been always made and, when they have been, they did not give univocal relationships, showing a strong dependence of the relationship with cultivar and environment, that limits considerably the relevance of such diagnostic tools in a large range of situations. Easier to use is the indirect estimation of crop NNI by remote sensing measurements. This method allows the estimation of both actual crop mass, through LAI estimation and crop N content, through crop chlorophyll content. The possibility to have repeated estimations of crop NNI during the period of vegetative growth would allow a dynamic diagnostic tool of crop N status. The coupling of indirect measurements of crop N status with dynamic models of crop growth and development should allow a very promising method for crop N diagnostics for decision tools in N fertilization.

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## 1. Introduction

Nitrogen (N) is often considered to be the most important limiting factor, after water deficit, for biomass production in natural ecosystems. In cropping and grassland systems, N fertilization practices can provide a sufficient N supply for plants to achieve the potential yield allowed by the actual climatic conditions. But because of climate variability, and in consequence of potential yield, farmers, to ensure that this potential yield is reached

each year, applied often quantities of N fertilizers larger than the quantity strictly required for achieving maximum yield. Another uncertainty leading farmers to apply a too large quantity of N is the unpredictable soil N supply according to soil type, previous crop management and climate of the year. So this great uncertainty in both plant N demand in relation to its growth potential and soil N supply incited farmers to adopt secure fertilization strategies that led to an increased risk of N leaching in most of the intensive cropping systems. Such a secure strategy has been encouraged for cereal crops by the fact that the penalty for an excess of N supply by lodging has been drastically reduced by the breeding and adoption of cultivars resistant to lodging and chemical treatments, and because N fertilizers were relatively

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cheap compared to the economic benefits from a maximised crop yield.

Nowadays, protection of soil water and air quality becomes a necessary constraint for agriculture, and the secure fertilization strategy cannot be longer used. In the hypothesis of a continuous increase of the energy price, N fertilizers will be more and more expansive. Moreover, considering global warming, the cost of N fertilizers should be evaluated in term of CO<sub>2</sub> equivalent released in atmosphere by fertilizer factories, and the emission of N<sub>2</sub>O from cropping systems should be also included. With the new Common Agriculture Policy in Europe, maximum profitability of farmers does not always correspond to maximum yield and the target yield of farmers can become significantly lower than the potential. So the new paradigm for N fertilization, instead of applying too much N to be sure to cover always potential crop N demand, should be to (i) determine crop N demand corresponding to different target yields, (ii) estimate soil N supply dynamics, and (iii) determine corresponding N application rate and timing. For achieving these objectives, it is necessary to develop theoretical models relating (i) plant growth dynamics and crop N uptake, (ii) soil N supply and climate, and (iii) crop N uptake and yield component formation. In a second step it would be necessary to derive from these models diagnostic tools usable within decision-making procedures allowing farmers to adapt their fertilizer management to their target yield and to environmental objectives according to the climate conditions. In a first part we develops concepts and theory for the determination of the critical crop N intake and plant N concentration, that allows the determination of a Nitrogen Nutrition Index related to plant and crop N status. In a second part, different operational methods for determination of NNI in fields are developed.

## 2. A conceptual framework to quantify plant growth and N uptake

### 2.1. Critical dilution curves: observation and theory

Even when there is ample supply of N, the N concentration in plants within dense canopies declines as they grow (Greenwood et al., 1986). This phenomenon has usually been interpreted as resulting from plant ageing and was related to plant phenology. Lemaire and Salette (1984a,b) and Lemaire et al. (1985) demonstrated that for grasses and lucerne the decline in plant N concentration (%N) was related to dry matter accumulation by stand (*W*) whatever the climatic conditions of the year or the species and genotype. This decline in %N was described by a negative power function called “dilution curve”:

$$\%N = aW^{-b} \quad (1)$$

When *W* is expressed in t ha<sup>-1</sup> and %N in percent, then coefficient *a* represents plant N concentration in percent when crop mass is 1 t ha<sup>-1</sup>. Coefficient *b* is dimensionless. Justes et al. (1994a) developed for wheat a statistical method for determining the critical plant N concentration at different times during the growth period from experimental data sets covering a large range of N application rates. Critical plant N concentration (%N<sub>c</sub>)

Table 1

Values of the coefficients of Eq. (2): N<sub>c</sub> = a<sub>c</sub>W<sup>-b</sup> for different crop species

Crop species	a <sub>c</sub>	b
Temperate grasses (C3)	4.8	0.32
Lucerne (C3)	4.8	0.33
Pea (C3)	5.1	0.32
Wheat (C3)	5.3	0.44
Rape (C3)	4.5	0.25
Rice (C3)	5.2	0.52
Tomato (C3)	4.5	0.33
Maize (C4)	3.4	0.37
Sorghum (C4)	3.9	0.39
Tropical grasses (C4)	3.6	0.34

is defined as being the minimum plant N concentration allowing maximum growth rate (Ulrich, 1952). So it was possible to determine a critical N dilution curve:

$$\%N_c = a_c W^{-b} \quad (2)$$

where a<sub>c</sub> is the critical plant N concentration for W = 1 t ha<sup>-1</sup>. Such an approach has been developed for several crop species: temperate grasses and lucerne (Lemaire and Gastal, 1997), tropical grasses (Duru et al., 1997), maize (Plénet and Lemaire, 2000), sorghum (Plénet and Cruz, 1997), rice (Sheehy et al., 1998), winter canola (Colnenne et al., 1998), pea (Ney et al., 1997), tomato (Tei et al., 2002). It is then possible to determine coefficients a<sub>c</sub> and *b* for these species as shown in Table 1.

Coefficient a<sub>c</sub> is highly different between C3 and C4 plants reflecting the different metabolic pathway, but is relatively constant within the same metabolic group. Coefficient *b* is a little bit more variable among species, the majority of them having a value comprise between 0.3 and 0.4 but without any detectable difference between C3 and C4 groups.

Greenwood et al. (1990) and Lemaire and Gastal (1997) developed a theory for explaining the dilution of N in growing plants. This theory is based on the assumption that plant mass *W* is composed of two compartments: W<sub>m</sub> the metabolic tissues involved directly in plant growth processes (photosynthesis and meristem activity) with high N concentration %N<sub>m</sub>, and W<sub>s</sub> the structural tissues involved in plant architecture with low N concentration %N<sub>s</sub>. Then:

$$W = W_m + W_s \quad (3)$$

and:

$$\%N = \frac{\%N_m W_m + \%N_s W_s}{W} \quad (4)$$

If we suppose that W<sub>m</sub> increases allometrically with *W*, then:

$$W_m = kW^\alpha \quad (5)$$

and then:

$$\%N = k(\%N_m - \%N_s)W^{\alpha-1} + \%N_s \quad (6)$$

This equation is similar to the empirical relationship of Eq. (1), the asymptotic value of %N being N<sub>s</sub> instead of 0, but owing to the low value of %N<sub>s</sub> estimated to 0.8 (Lemaire and Gastal, 1997) the difference between the two curves is not important

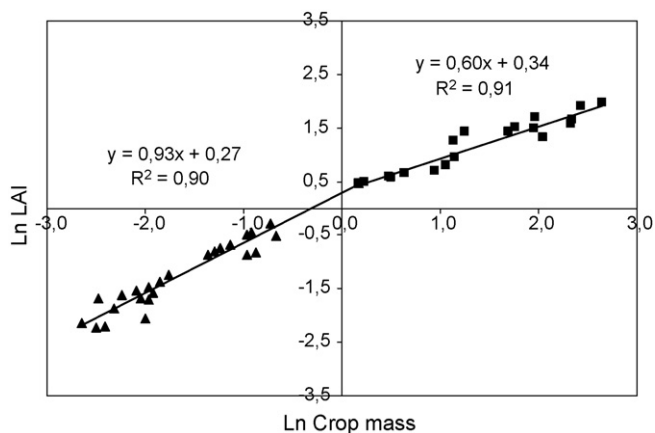


Fig. 1. Relationships between  $\ln(\text{LAI})$  and  $\ln(W)$  for winter wheat for crop mass  $<$  or  $>$  to  $1 \text{ t ha}^{-1}$  (data from Jeuffroy and Bouchard, 1999). Coefficient  $\alpha$  of the Eq. (5) is equal to the slope of the regression.

within the range of value for  $W$  ( $1\text{--}20 \text{ t ha}^{-1}$ ). In this condition, the theoretical value of coefficient  $\alpha - 1$  should be close to the value of coefficient  $b$ . If we postulate that  $W_m$  scale for plant area (Hardwick, 1987) it is then possible to establish an allometric relationship between LAI and  $W$  directly from Eq. (5):

$$W_m = p \text{LAI} \quad (7)$$

and:

$$\text{LAI} = \frac{k}{p} W^\alpha \quad (8)$$

where  $k/p$  being the leaf area per unit of plant mass (LAR: leaf area ratio) when  $W = 1 \text{ t ha}^{-1}$ , it has been called “intrinsic leafiness” (Lemaire et al., 2007) denoting the intrinsic plant architecture. If we assume that  $W$  scale with plant volume and with a constant plant mass per unit volume in first approximation, then the coefficient  $\alpha$  in Eq. (5) should be equal to  $2/3$  if the growth of plant is isometric, i.e. if the relative growth of plant is the same in the three dimensions.

Fig. 1 shows the relationship between  $\ln(\text{LAI})$  and  $\ln(W)$ . The slope of the relationship is then an approximation of coefficient  $\alpha$  according to Eq. (8). As shown by Lemaire et al. (2007), this slope decreases from the early period of growth after sowing when  $W$  is less than  $1 \text{ t ha}^{-1}$ , that corresponds to near isolated plants, to period after  $W = 1$  when canopy becomes closed. These authors showed that for different crops as lucerne, canola, sunflower, wheat, rice, maize and sorghum the value of  $\alpha$  is not different from  $0.95$  for  $W < 1 \text{ t ha}^{-1}$  and not different from  $0.6$  for  $W > 1 \text{ t ha}^{-1}$ . It implies that for early crop growth stages, when plants are nearly isolated, leaf area expands about  $5\%$  less than biomass accumulation in relative term, leading to a very small N dilution (value of coefficient  $b$  close to  $0.05$ ) while, when plants are growing in a dense stand, competition for light induces a near isometric growth leading to severe N dilution (value of coefficient  $b$  near  $0.3\text{--}0.4$ ).

As shown in Table 1, the value of coefficient  $b$  which is an approximation of coefficient  $\alpha - 1$  in dense canopy conditions ( $W > 1$ ) is around the theoretical value of  $0.33$  corresponding to an isometric growth for most of the species studied whatever

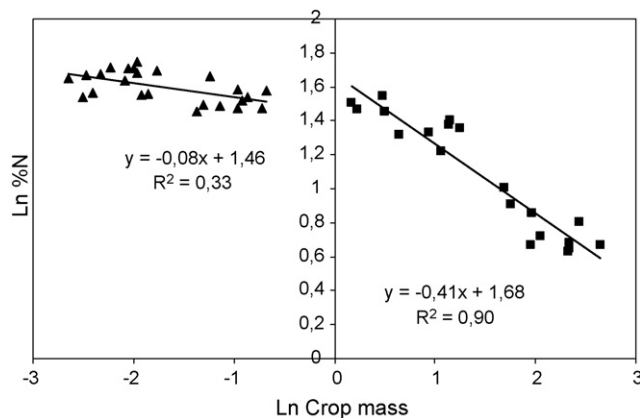


Fig. 2. Relationships between  $\ln(\%N)$  and  $\ln(\text{crop mass}, W)$  for winter wheat for crop mass  $<$  or  $>$  to  $1 \text{ t ha}^{-1}$  (data from Jeuffroy and Bouchard, 1999). Coefficient  $b$  of Eq. (2) is equal to the slope of the regression.

their group C3 or C4 and monocot or dicot. The two exceptions are wheat and rice with values of  $b$  of respectively  $0.44$  and  $0.52$  corresponding to values of  $\alpha$  less than  $2/3$  that indicates that for these two crops the relative growth in the third dimension (height and thickness) should be higher than relative growth in area.

Fig. 2 shows that the values of coefficient  $b$  for both growth periods before or after  $W = 1$  are close to the corresponding values of  $1 - \alpha$  deduced from Fig. 1. This strong correlation between LAI development and N dilution has been confirmed for all the species mentioned above (Lemaire et al., 2007) and seems to be a very general feature. So the dilution of N within plant seems to be accelerated when canopy becomes closed. Before this stage the value of  $\alpha$  close to but less than  $1$  indicates that plants tend to maximise their growth in area that leads to a low N dilution, while after this stage, the value of  $\alpha$  close to  $2/3$  indicates an isometric growth leading the plant to grow in the third dimension (height) as a result of competition for light (Pons et al., 1989). So the decreasing plant N concentration as canopy develops is the result of two processes: (i) a decrease of the leaf area ratio (LAR: leaf area per unit of plant mass) of plants because the plant invests a greater proportion of biomass in structural compartment ( $W_s$ ) relatively to leaf area ( $W_m$ ), that allows the plant to grow in height for reaching light, and (ii) a decrease of N content per unit of leaf area of shaded leaves that corresponds to an optimisation of N allocation in relation to light distribution, that allows an optimisation of the canopy photosynthesis (Hirose and Werger, 1987). As a result of these processes, for crop maintained in non-limiting N supply, despite the pronounced plant N concentration decline, the N content of well illuminated leaves at the top of the canopy remains more or less constant (Gastal and Lemaire, 2002).

## 2.2. Crop N demand

The Eq. (1) can be transformed by multiplying the two members by  $W$ , leading to a relationship between crop N uptake and crop mass accumulation:

$$N = a' W^{1-b} \quad (9)$$

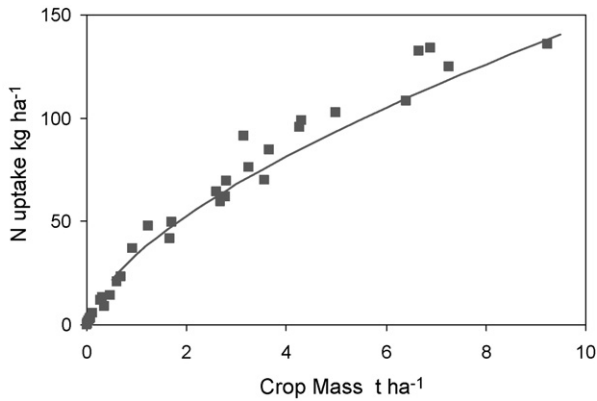


Fig. 3. Relationship between N uptake and crop mass for maize. Points represent data obtained in subtropical conditions in Australia without limitation of N (after Lemaire et al., 2007). The line represents the critical N uptake curve determined by Plénet and Lemaire (2000) for maize growing in temperate conditions in France:  $N = 34W^{0.63}$ .

with N, crop N uptake in  $\text{kg ha}^{-1}$  and  $a'$ , the quantity of N taken up for  $W = 1 \text{ t ha}^{-1}$ . So it is possible to determine the critical N uptake ( $N_c$ ) as the minimum N uptake corresponding to maximum crop mass directly derived from Eq. (2). So as shown by Fig. 3, the dynamic of crop N demand is directly deducible from the dynamic of crop mass accumulation which is in turn determined by temperature, radiation, water availability and genotype.

Coefficients  $1 - b$  and  $\alpha$  being very close and varying simultaneously from period of near isolated plant to period of close canopy as shown by Lemaire et al. (2005) on lucerne and Lemaire et al. (2007) on a large range of crop species including C3 and C4 monocots and dicots, it was then possible to establish a strict proportionality between crop N uptake and crop LAI during all the vegetative growth period from seedling emergence to maximum LAI of the crop as illustrated by Fig. 4. After crops reach the maximum LAI, grain filling processes and N remobilisation from senescent leaves are the two processes dominating N economy in major annual crops, and crop N demand for uptake is determined by the balance between grain demand in N and the supply of N by remobilisation from leaves. But at this stage

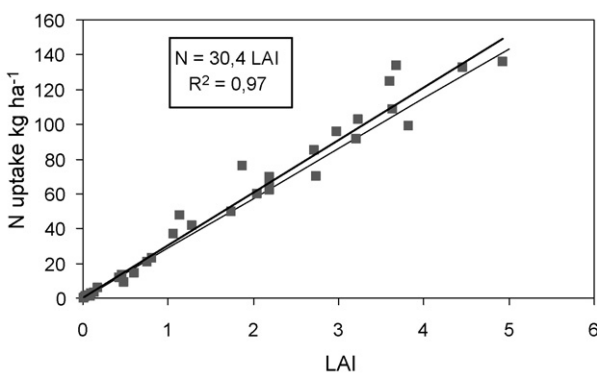


Fig. 4. Relationship between N uptake and LAI for maize growing in subtropical conditions in Australia (after Lemaire et al., 2007). The dotted line represents the relationship established by Plénet and Lemaire (2000) in temperate conditions in France:  $N = 28.9\text{LAI}$ .

the number of grains and then the potential yield is already determined (Jeuffroy and Bouchard, 1999).

### 2.3. Regulation of crop N uptake by both plant growth and soil N supply

The theory and observations illustrated above indicate that N uptake seems to be regulated by plant growth itself. Lemaire et al. (2007) discussed if leaf area expansion or biomass accumulation is the control variable. They concluded that in fact both interact, biomass accumulation and leaf area expansion being the two faces of a same regulation process. In a steady state N supply condition, plant N uptake is feed back regulated by shoot signals, with a positive signal from photosynthetic C supply and a negative one from reduced N re-circulation to the roots (Cooper and Clarkson, 1989; Ismande and Touraine, 1994; Lejay et al., 1999; Touraine et al., 2001; Forde, 2002). The relationship between N uptake and LAI during the vegetative growth period can thus be explained by the fact that LAI expansion provides larger C supply to roots, and also increases the N storage capacities within leaves as Rubisco (Millard, 1988) that avoids the depletion of N uptake by re-circulating reduced N in the phloem. That leads to proportionality between N uptake and LAI for most of species (Lemaire et al., 2007), but the slope of the relationships, i.e. the N uptake per unit LAI is variable across species or genotypes according to their own intrinsic morphology or “leafiness” as expressed by LAR or Leaf/Stem ratio. So, taking into account that leaf area expansion is not the only way by which plants can store reduced N, but that stem growth (or leaf thickness increase) is also a mean for the plant to store reduced N, a more stable relationship is obtained across species, genotype and environments when N uptake is related to crop mass. Nevertheless this relationship is not linear (Eq. (9) and Fig. 3) because the N uptake per unit of W accumulated decreases as the leaf area per unit crop mass decreases, which determines the N dilution effect. So, even though the relationship between N uptake and LAI is simpler (only one coefficient of proportionality) than the relationship between N uptake and W, the last one is more robust and allows the determination of crop N demand, i.e. the minimum N uptake for achieving maximum crop mass in a given environment.

In fact, in a variable N supply condition, plant N uptake is co-regulated by both crop growth rate potential and N availability in soil. Devienne-Barret et al. (2000) proposed a model where the N uptake rate  $dN/dt$  is determined both by potential crop growth rate  $dW/dt$  and the concentration of mineral N in soil. Such a co-regulation is illustrated on Fig. 5.

For each N supply level corresponds a N uptake–crop mass trajectory with time which is covered more or less rapidly depending on the potential crop growth rate as determined by climatic conditions and genotype. Such a co-regulation leads to the fact that crop N uptake from non-fertilized crops is determined by both soil N supply level and potential crop growth rate (difference between As and Bs). So a species or genotype with a high potential growth rate should uptake a greater quantity of N from a given soil than species and genotype having a lower potential growth rate. The critical N uptake–crop mass trajec-

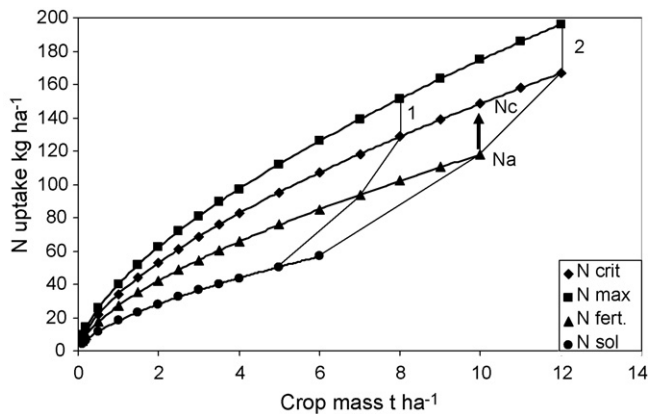


Fig. 5. N uptake–crop mass trajectories for different steady state N supplies: critical N uptake (crit.), maximum N uptake (max), non-fertilized (soil) and sub optimal N application (fert.). (1) and (2) represent either (i) two growth stages of the same crop, or (ii) two crops having different growth rates, or (iii) an environmental effect. The lines represent the responses curves to increased N supply.  $N_a$  is the actual N uptake of the limiting fertilized treatment,  $N_c$  is the corresponding critical N uptake at similar crop mass.

tory is defined by Eq. (9) and for a given species is determined by the value of coefficients  $a$  and  $b$  of Table 1. This corresponds to the ideal trajectory for maximising crop growth with the minimum N uptake. The curve corresponding to the maximum is not well known. It should correspond theoretically to the maximum capacity of a crop to accumulate N. Justes et al. (1994a) for wheat and Plénet and Lemaire (2000) for maize have established an empirical curve as the maximum observed N uptake within a large experimental data set. But these curves have no physiological support.

### 3. Relationships with the concept of N productivity

The concept of plant nitrogen productivity has been introduced to interpret the dependency of plant growth on internal nitrogen (Agren, 1985). The variation of plant internal nitrogen can be due to either variations in external nitrogen concentration (Agren and Ingestad, 1987), or the decrease of plant N concentrations with increasing plant mass in non-limiting external nitrogen supply (Poorter et al., 1990). Plant nitrogen productivity (NP) is then defined as the increase in plant dry matter per unit of time and per unit of plant nitrogen content (Agren, 1985):

$$NP = \frac{dW}{Nd t} \quad (10)$$

where  $dW/dt$  is the plant growth rate, and  $N$  is the plant nitrogen content.

Garnier et al. (1995) showed that NP can be decomposed in two components: (i) the leaf nitrogen productivity,  $NP_L$ , that is the dry matter production per unit of leaf N content, and (ii) the fraction of plant nitrogen present in the leaves,  $f_{NL}$ :

$$NP = f_{NL} NP_L \quad (11)$$

It was argued that  $NP_L$  is likely to depend on Photosynthetic Nitrogen Use Efficiency (PNUE) (Konigs, 1990) defined

as the ratio between the rate of photosynthesis and leaf nitrogen concentration (Field and Mooney, 1986).

Re-arrangement of Eq. (10) gives:

$$NP = \frac{dW}{N\% W dt} \quad (12)$$

Where  $N\%$  is the plant N concentration and  $dW/Wdt$  is the plant Relative Growth Rate (RGR). Ingestad (1979) found that plant RGR was linearly related to plant N concentration. Greenwood et al. (1991) showed that under non-limiting N conditions the decrease in plant  $N\%$  as described by the critical N dilution curve (Eq. (2)) parallels the decrease in RGR. So as a consequence the plant N productivity remains constant during the time course of crop growth. These authors showed that within each plant metabolic pathway, C3 or C4, there is very little variation in the relationship between RGR and plant  $N\%$  for a large range of cultivated species. Such a result is due to (i) the high N requirement for photosynthetic apparatus ( $\%N_m$  in Eq. (4)), (ii) the low N requirement in structural tissues ( $\%N_s$  in Eq. (4)), and (iii) the similarity in PNUE of species within the same metabolic group.

## 4. Nitrogen Nutrition Index a basic tool for crop N status

### 4.1. Determination of NNI

As shown in Fig. 5, the critical N uptake curve separates situations where N supply is limiting for crop mass accumulation from situations where N is accumulated in excess without any supplemental increase in crop mass. For a given situation and at any time course of the crop growth period, it is possible to determine a Nitrogen Nutrition Index (NNI) as the ratio between the actual crop N uptake ( $N_a$ ) and the critical N uptake,  $N_c$ , corresponding to the actual crop mass  $W_a$  (Eq. (13)), provided the critical N uptake of the crop species has been determined. NNI can be also determined directly from actual plant N concentration and dilution curves:

$$NNI = \frac{N_a}{N_c} = \frac{\%N_a}{\%N_c} \quad (13)$$

Values of NNI close to 1 indicate that at the date of the determination of  $N_a$  or  $\%N_a$  the crop were in situation of non-limiting N supply. Values more than 1 indicate a luxury consumption of N. Values lower than 1 indicate a N deficiency, the intensity of which can be estimated by the value of the NNI: a value of 0.6 indicating that crop N availability was only 60% of the critical level. Such an index of crop N status has been used by Lemaire and Meynard (1997) as a diagnostic tool for analysing *a posteriori* agronomical data from field experiments or farm observations in order to explain variations in yield by differences in crop N status.

## 5. Are NNI during vegetative phase of grain crops related with grain yield components?

NNI is in fact an estimation of an instantaneous crop N status at the date where determination of  $N_a$  (or  $\%N_a$ ) has been made.

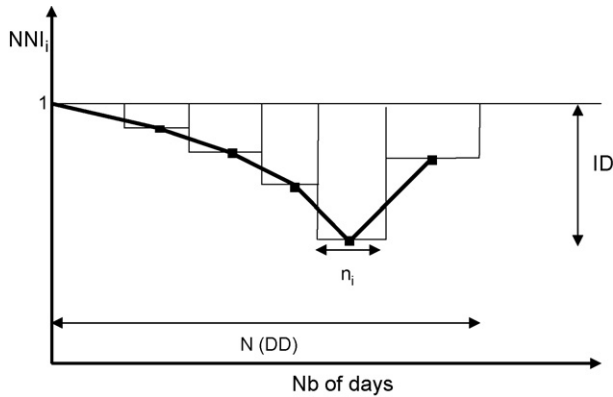


Fig. 6. Estimation of  $NNI_{int}$  from sequential determination of  $NNI_i$  according to Eq. (11):  $NNI_{int} = 1/N \sum NNI_i n_i$ . DD represents the duration of the period of N deficit, and ID represents the intensity of N deficit.

The N uptake–crop mass trajectories of Fig. 5 are theoretical ones because they suppose that the crop N supply level remains at a steady state all along the crop growth period. In the reality, a crop can experience changing N supply conditions according to soil N mineralization activity, timing of N-fertilizer application or exhaustion of soil mineral N by crop itself. As grain yield formation depends on the conditions occurring during the period of elaboration of the yield components (and thus during a long period before grain set), it is difficult to infer directly the yield of a crop from its NNI at a given period. For example on wheat, a low NNI occurring during the tillering period can lead to a low number of stems bearing ears, but a higher NNI later during the stem elongation period can lead to an increased number of grains per ear, compensating the low ear number. Nevertheless, the time-course change of NNI during the vegetative period, which is the period of grain set, has a great effect on grain number, and thus grain yield.

An integrated NNI can be obtained by the weighted mean of NNI during the vegetative period as illustrated in Fig. 6, each interval on the X-axis representing the duration, expressed in day or in degree-days. Lemaire and Gastal (1997) showed that it was possible to establish a linear relationship between the  $NNI_{int}$  and the relative biomass accumulation as expressed as the ratio between actual biomass ( $W_a$ ) and maximum biomass ( $W_{max}$ ):

$$\frac{W_a}{W_{max}} = K(NNI_{int} - NNI_0) \quad (14)$$

where  $K$  is the response of the crop to increment in its average N supply status estimated by  $NNI_{int}$  and  $NNI_0$  is the minimum crop N status to allow plant growth. This minimum corresponds theoretically to  $\%N_s$  as determined by Eq. (5). Another way to take into account the time-course change of NNI was proposed by Jeuffroy and Bouchard (1999) to analyse the effect of N deficiency on grain number for wheat. These authors characterized the N deficiency period of a wheat crop by both its length (deficit duration, DD) and its intensity (ID) by means of  $1 - NNI$  minimum observed value and calculated an integrated index of crop N status by the mean of the product  $ID \times DD = IDD$  that represents about twice the area between the curve of  $NNI_i$

dynamic and the horizontal line  $NNI_i = 1$  as shown on Fig. 6. So in fact IDD is about twice  $NNI_{int}$ . They showed that IDD explained 96% of the variation in grain number of wheat within a large experimental data set, while NNI at anthesis explained only 92% of this variation as already mentioned by Justes et al. (1997a,b). Plénet and Cruz (1997) have shown that grain number on maize is highly correlated to  $NNI_{int}$  estimated during the period from seedling to 20 days after silking, the weight of thousand grains being also correlated, leading to a strong effect on grain yield, the slope of the regression between relative grain yield ( $Y_a/Y_{max}$ ) and  $NNI_{int}$  being 1.15, that implies that any reduction of  $NNI_{int}$  of 0.1 lead to a reduction of grain yield of 11.5%. On oilseed rape also there is a good relationship between seed number and the values of NNI during the period of seed set (Jeuffroy et al., 2003). On pea, N deficiencies can occur in the fields, due to either insect damage by *Sitona lineatus* L, or by compacted soil structure (Doré and Meynard, 1995). In these cases, a good relationship was also observed between seed number and the crop NNI at the beginning of anthesis (Doré et al., 1998).

So it is clear that NNI is a good basic tool for analysing actual plant N status in crops but several constraints avoid the practical use of this indicator as an operational diagnostic tool for N fertilizer management. First, as demonstrated above, only one determination of NNI during growth period does not allow direct inference of  $NNI_{int}$ , earlier is the NNI determination for prognostic purpose, worst is the correlation with  $NNI_{int}$  and crop yield components owing to the variation in soil N supply with time. Nevertheless determination of NNI would offer a diagnostic tool for decision rule in N fertilizer management: a NNI measured at a given date just equal or greater than 1 can lead to suppress, reduce or delay a fertilizer application, until NNI drops below 1 and in the reverse, a  $NNI < 1$  could lead to anticipate or to major an application depending on the intensity of the N deficiency recorded.

## 6. Practical tools for N status diagnostic and N fertilization management

It is clear that determination of NNI at given intervals during the vegetative growth period of crops could help for optimizing the timing and the rate of N fertilizer applications in order to adjust as precisely as possible N supply to crop N demand corresponding to target yield. But, such frequent measurements of NNI, which are possible in experimental fields, are not feasible in farm fields. The determination of NNI requires very time consuming procedures: (i) determination of the actual crop mass ( $W_a$ ) with representative sampling areas, weighting fresh mass, sampling for determination of dry matter content, oven-drying, weighting, sample grounding, and (ii) determination of actual plant N concentration by analytical procedure in laboratory. These procedures are out of the expertise of farmers and of the time they could use to obtain the information. So NNI must be considered as a basic indicator of plant N status, but it is necessary to develop indirect methods for estimating its value or at least the two components  $W_a$  and  $\%N_a$ .

### 6.1. Nitrate concentration in sap

Various authors (Scaife and Stevens, 1983; Gonzalez-Montaner, 1987) have pointed out the interest of measuring plant nitrate concentration to evaluate the N nutrition status of vegetables or cereals. Plant sap from stem bases can be extracted by pressure and then nitrate concentration can be immediately estimated by the use of rapid test strips. Justes et al. (1994b) and Justes et al. (1997a,b) developed an integrated diagnosis tool based of nitrate sap concentration for N management decision on winter wheat and maize. This method is based on several steps: (i) calculate the overall fertilizer requirements of the crop using the balance-sheet method (Machet et al., 1990), that gives the total N rates; (ii) apply a reduced amount of fertilizer ( $X - 40 \text{ kg ha}^{-1}$ ) by splitting application at tillering and at the beginning of stem elongation; (iii) measure nitrate concentration in the base of main stem to detect N deficiency at three stages during stem elongation (1st node, 2nd node and flag leaf emergence); (iv) apply or omit the last dressing of  $40 \text{ kg ha}^{-1}$  or more depending on the stage at which N deficiency occurs and its intensity. For application the relationship between nitrate concentration in sap and plant NNI has to be calibrated. Justes (1992) and Justes et al. (1997a,b) showed that the relationship between these two variables is very complex and not univocal. Nitrate concentration within stem base depends on many factors such as phenology stage, cultivar, temperature and solar radiation and not only on plant N status. Nevertheless despite the very weak correlation between sap nitrate concentration and NNI, it was possible to show that when  $\text{NNI} < 1$ , nitrate concentration in sap is never higher than  $1 \text{ g l}^{-1}$ , while for  $\text{NNI} > 1$  sap nitrate concentration can vary from 1 to  $10 \text{ g l}^{-1}$  whatever the value of NNI. So this test cannot be used as a quantitative estimation of crop N status. It can distinguish situations with probable N deficit from situations with probable satisfactory N status and then it would be possible to adapt fertilization strategy in consequence.

### 6.2. Upper layer leaf N content

The theory developed above for explaining N dilution with plant growth in dense canopy shows that the decrease of plant N concentration is the result of two processes: (i) the decline in plant LAR as crop mass increases, and (ii) the preferential allocation of N to the well illuminated upper layer of leaves as canopy develops. Therefore, Lemaire et al. (1997) suggested that while plant N concentration declines with crop mass accumulation the N content per unit leaf area within the upper layer of the canopy would remain more or less constant in a steady state N supply condition. Gastal et al. (2001) established that for grass stands, the N concentration of the upper layer of leaves was constant through the regrowth period and correlated well with the NNI. Then Farrugia et al. (2004) using this correlation developed a method of diagnostic of grassland N status. The procedure consists in harvesting the apical part of the first well developed leaf of sampled tillers in the field plot and to measure in laboratory leaf N concentration. Sampling individual tillers at random can provide a good average estimation of plant N status

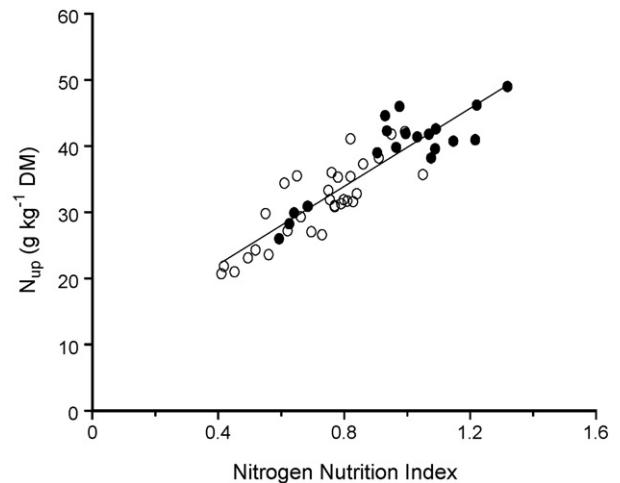


Fig. 7. Relationship between NNI of the swards and N concentration of the lamina at 10 cm top of the canopy ( $N_{\text{up}}$ ). The two symbols represent two series of experimental fields. (after Farrugia et al., 2004).

at field level without having to measure sward biomass. Fig. 7 represents the relationship between N concentration of upper layer of leaves and NNI of different grassland field plots with a large range of N supply conditions. These grassland fields were dominated ryegrass swards but with variable proportion of different other species. Gonzalez-Dugo et al. (2005) confirmed that this method hold for different grass species such as tall fescue, perennial ryegrass and cocksfoot.

This method should allow the evaluation of N status of individual plants within a plurispecific stand owing to the fact that individual plants of each species can be sampled separately for their determination of their own N status. Such a procedure should be highly interesting for grass-legume mixtures in order to know if companion grass is under limiting or non-limiting N supply.

The NNI estimated by Farrugia et al. (2004) have been compared with soil test indicating the quantity of mineral N in soil within the respective field plots (Scholefield and Titchen, 1995). For plots having more than  $20 \text{ kg ha}^{-1}$  of mineral N, the NNI estimated by the N concentration of the upper leaf layer was always higher than 1, indicating a non-limiting N nutrition and even a luxury N consumption. For plots having less than  $20 \text{ kg ha}^{-1}$  of soil mineral N, NNI varied between 0.4 and 1.4 indicating that no correlation existed between plant and soil tests. In fact soil mineral N content can be low for two reasons (i) because N mineralization is low and then plants suffer from N deficit, or (ii) because plant N uptake is high and mineralized N cannot accumulate in soil, but with no systematic N deficiency of the plant. This result demonstrates that soil N supply is a very dynamic process and then we have to estimate N fluxes and not N concentration. The plant test appears then more accurate than soil test for determining fertilizer application management.

### 6.3. SSNM decision tool system

In the Site-Specific Nutrient Management (SSNM) approach, fertilizers are applied using the following principles to achieve

high yield and high efficiency of plant use in rice (Dobermann et al., 2004): (i) apply only a moderate amount of fertilizer N to young rice within the 14 days after transplanting (DAT) or 21 days after sowing (DAS), when the need of the crop for supplemental N is small; and (ii) apply fertilizer N after 14 DAT or 21 DAS based on the crop's need for supplemental N, as determined by leaf N status. The leaf color chart (LCC) is a tool that could be used for assessing leaf N status and the crop's need for N. The leaf color chart (LCC) is an easy-to-use and inexpensive diagnostic tool for monitoring the relative greenness of a rice leaf as an indicator of the plant N status (Alam et al., 2005; Witt et al., 2005).

Leaf N status of rice is closely related to photosynthetic rate and biomass production, and it is a sensitive indicator of changes in crop N demand within a growing season. So the SSNM system is based on the same principle as the diagnostic of upper layer leaf N content (see Section 6.2) provided that leaf color chart would be well correlated to leaf N content and then to crop NNI.

#### 6.4. Chlorophyll meter

Different portable systems (©Hydro-N-Tester of Yara, or ©SPAD of Minolta) allow the measurement of chlorophyll content of leaves based on leaf transmittance or leaf reflectance in specific waves bands. It is then supposed a good relationship between the chlorophyll content and the leaf N content either per unit mass basis or per unit leaf area basis. Peng et al. (1996) showed that chlorophyll meter measurements were correlated with leaf colour chart estimations (see above 4.2). A lot of works tried to predict crop yield from the data obtained by these instruments during the time course of crop growth and development. But these predictions were in general relatively poor because the results were highly dependant of the year and the cultivar (Matsunaka et al., 1997; Le Bail et al., 2005). Others tried to use these measurements to estimate nitrogen fertilizer requirements (Piekelek and Fox, 1992; Feibo et al., 1998).

Indirect estimation of leaf N concentration by the mean of chlorophyll meter could be an interesting way for estimating upper leaf layer N concentration and then NNI, owing to the strong correlation between these two last variables. Nevertheless, Peng et al. (1993) showed that estimation of leaf N concentration was very sensitive to variations of specific leaf weight according to environment and cultivars. Moreover, the nitrogen contained in the chlorophyll molecules represents only 2% of the total nitrogen of the leaf which is dominated by N of photosynthetic proteins (Lawlor et al., 1997). One should therefore be aware that the experimental relationships which may be established between chlorophyll and nitrogen content remain highly empirical (Houlès et al., 2007). A large number of authors find in fact a good correlation between chlorophyll content and leaf N content measurements (Piekelek and Fox, 1992; Feibo et al., 1998; Reeves et al., 1993; Matsunaka et al., 1997). But these relationships are highly variable from a study to another according to environmental conditions and genotypes. So despite large use in experimental work and in extension services chlorophyll meter are a poor indicator of NNI as showed by Houlès et al.

(2007). Even if the direct indications of SPAD or HNT are poorly correlated to NNI, it is nevertheless possible to use these indicators in relative term: for example as the ratio of SPAD of a limiting N crop and the SPAD of a non-limiting N check area within the field crop (receiving an ample N application). This method avoids of effect of genotype and environment on the regression between SPAD and NNI as shown by Debaeke et al. (2006).

#### 6.5. Remote sensing

Several authors used remote sensing based on reflectance for estimating chlorophyll content on an area basis at the scale of the canopy (Baret and Fourty, 1997; Clevers, 1999; Jongschaap and Booil, 2004). Dumont and De Baerdemaeker (2001) suggest working at integrated scale, i.e. not in terms of concentration but in terms of the quantities of chlorophyll and nitrogen present in the canopy in order to remove the difficulty for converting contents from an area basis to a mass basis (Roderick et al., 1999).

Then Houlès et al. (2007) compared three methods for evaluating crop N status with remote sensing measurements:

- (i) NNI is directly estimated by empirical relationships with chlorophyll concentration within the canopy as measured by canopy reflectance.
- (ii) Leaf N concentration is estimated by empirical relationship with chlorophyll concentration ( $C_{ab}$ ), and crop LAI is measured by remote sensing, and then actual crop mass ( $W_a$ ) is estimated through an empirical relationship between LAI and  $W_a$  (see Eq. (8) above). So critical N concentration ( $\%N_c$ ) can be calculated using Eq. (2) and then NNI can be estimated.
- (iii) The quantity of chlorophyll per unit soil area ( $QC_{ab}$ ) is deduced directly from remote sensing measurements and is related to the quantity of N within the canopy per unit soil area ( $N_a$ ). LAI and  $W_a$  are estimated as in (ii) that allows the calculation of critical N uptake ( $N_c$ ) using Eq. (9). Then NNI can be calculated.

These authors demonstrated that the third method gave the best prediction. So it appears possible to use remote sensing either through satellite measurements or with proximal measurements to give an accurate estimation of the crop N status. Moreover such an estimate can be repeated in time and in space that allows very precise information on the spatio-temporal dynamics of crop N status that is very useful for precision agriculture.

#### 6.6. Coupling with crop models

Methods of indirect estimation of crop N status as presented above using instantaneous indicators such as HNT or SPAD index can be advantageously coupled with dynamics crop models in order to provide accurate prediction of crop growth and development, and then of yield according to N fertilization management decisions. NNI of a crop can be predicted by using



crop models such as AZODYN (Jeuffroy and Recous, 1999) or STICS (Brisson et al., 2002) before the date of N application. Then the predicted value can be used for identifying field plots in which N fertilizer must be applied. Naud et al. (2007, 2008) showed that the prediction of NNI by the model AZODYN can be highly improved by assimilation of data provided by earlier measurements or estimations of some crop variables such as LAI, biomass or chlorophyll meter. So coupling crop models with estimated or measured crop parameters could allow a better estimation of the crop N status at the target periods for decision for fertilizer application than using separately these two approaches.

## 7. Conclusion

Theory allows the definition of a method of determination of crop N nutrition status during vegetative growth period, i.e. during which plant accumulates N within leaf and stem tissues. The Nitrogen Nutrition Index as presented above represents a way for crop N diagnostics. For most of annual grain crops the N accumulated in crop biomass at the end of this period, i.e. at anthesis determines (i) the potential yield through the number of grains, and (ii) about the 2/3 third of the amount of N available for grain filling. The about 1/3 remaining being absorbed during the grain filling period are more sensitive on protein content of grains. So as a diagnostic tool for N fertilization management, NNI can be determined during all the vegetative growth period. Close relationships have been established between NNI during vegetative period and the yield components on several crops, so it appears possible to monitor crop N status by adequate N application rate and timing in order to adapt crop N uptake to the target yield. Nevertheless the determination of NNI is very time consuming and cannot be made all along the vegetative growth period in practical conditions of farms. Several indirect plant N concentration indicators have been proposed in literature. These indicators have to be validated and calibrated with the NNI, owing its strong ecophysiological significance. Most of these indicators are only lightly related to NNI because of a non-univocally relationship depending too much on cultivars and environmental conditions. Nevertheless a new procedure of indirect measurement of both crop LAI and crop N content would allow the estimation of crop NNI by remote sensing. Because remote sensing measurements are not invasive and can be repeated several times along the growth period, information obtained on crop N status dynamics can be used for decision making in N fertilizer management. According to Meynard et al. (1997), the qualities required for indicators of nitrogen diagnostic should be: (i) specificity: if the indicator varies only with nitrogen nutrition; (ii) sensitivity: if the indicator reacts rapidly to any change in plant N nutrition status; (iii) memory: the capacity of indicators to give information about the history of the stand; (iv) predictive value: the possibility to infer future elements of crop behaviour. NNI seems to have all these qualities, but when NNI is estimated through indirect methods some of these qualities can be deteriorated. So a trade-off exists between operational use and quality of N diagnostic tools.

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