Plant Analysis as a Tool to Determine Crop Nitrogen Status

– Towards Leaf Area Based Measurements

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Abstract

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An effective plant nutrient management strategy optimises nitrogen (N) use efficiency for minimised environmental impact, while ensuring an optimum N status of the crop for good product quality and maximum growth. Soil or plant analysis can be used to evaluate the strategy; however the use of plant analysis for this purpose has been limited. One reason is lack of reliable reference values for the critical concentration needed for optimal growth. This study builds on theories that relate ontogenetic changes in the critical N concentration to changes in the relation between mass and surface area of the entire plant and of individual leaves. Through the establishment of critical N concentrations on the basis of these theories, some of the drawbacks hitherto experienced with plant analysis, such as difficulties in defining growth stage or plant part to sample, can be avoided.

The aim of this thesis was to establish critical N concentrations for white cabbage (*Brassica oleracea* L. var. *capitata* L. f. *alba* D.C.) on the basis of these theories. Multi-N-rate and multi-harvest experiments were conducted in the field and in a climate chamber.

The results showed that the critical N concentration declined at the same rate (−0.33) as the plant's leaf area ratio (leaf area divided by plant mass), which is in agreement with the 2/3-Power rule or "skin-core" hypothesis. The critical N concentration (% of DM) on a whole plant basis was estimated to 4.5 ($W<1.5$ t ha^{-1}) and to $5.1W^{-0.33}$ ($W>1.5$ t ha^{-1}), where W is weight per unit area of plant dry matter exclusive of roots. Moreover, it was concluded that the unshaded horizontally orientated leaves of cabbage can be used for leaf area based plant analysis of individual leaves. The critical N concentration of these leaves expressed on an area basis was found to be 3.7 g N m^{-2}, while that for the whole plant N on a leaf area basis was 4.7 g N m^{-2}. The ratio of these two critical concentrations, 0.8, was similar to the leaf N ratio (leaf N/whole plant N) of young plants before self shading occurs.

Key words: critical nitrogen concentration, ontogenetic decline, leaf area ratio, leaf nitrogen ratio, specific leaf nitrogen

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Contents

Preface

Papers I –IV

The thesis is based on the following papers, which are referred to by their Roman numerals:

I. Göran Ekbladh, Ernst Witter, Tom Ericsson, 2007.
Ontogenetic decline in the nitrogen concentration of field grown white cabbage – Relation to growth components
Scientia Horticulturae, 112, 149-155.

II. Göran Ekbladh, Tom Ericsson, Ernst Witter
Ontogenetic changes in the nitrogen concentration of white cabbage — I. Relation to relative growth rate and its components
(Manuscript)

III. Göran Ekbladh, Tom Ericsson, Ernst Witter
Ontogenetic changes in the nitrogen concentration of white cabbage — II. Leaf-area-based assessments on individual leaves (Manuscript)

IV. Göran Ekbladh, Ernst Witter
Determination of the critical nitrogen concentration of white cabbage (Manuscript)

Göran Ekbladh's contribution to the papers included in this thesis was as follows:

I Data analysis, wrote manuscript with contributions from Witter and Ericsson.

II Planned the experiment together with Ericsson, work in climate chambers, data analysis, wrote manuscript with contributions from Ericsson and Witter

III Planned the experiment together with Ericsson, work in climate chambers, data analysis, wrote manuscript with contributions from Ericsson and Witter

IV Planned the experiment, field work, data analysis, wrote manuscript with contributions from Witter

Abbreviations

DAT	Days after transplanting
DM	Dry matter
GR	(Absolute) growth rate [g $plant^{-1}$ day^{-1}]
LA	Leaf area [m^2]
LAI	Leaf area index (leaf area/ground area)
LAR	Leaf area ratio (LA/W) [cm^2 g^{-1}]
LN	Accumulated leaf nitrogen [g N $plant^{-1}$]
LNC	Leaf nitrogen concentration (LN/LW, leaf weight) [Percent of DM]
LNC_a	Leaf nitrogen concentration on an area basis (LN/LA) [g N m^{-2}]
LNP	Leaf nitrogen productivity (GR/LN) [g $plant^{-1}$ day^{-1} g^{-1}]
LNR	Leaf nitrogen ratio (LN/PN) [fraction]
N	Nitrogen
NAR	Net assimilation rate (GR/LA) [g $plant^{-1}$ day^{-1} m^{-2}]
NDF	Neutral detergent fibre [g g^{-1}]
NNI	Nitrogen nutrition index [fraction]
NP	Nitrogen productivity (GR/PN) [g $plant^{-1}$ day^{-1} g^{-1}]
PFD	Photon flux density [μmole m^{-2} s^{-1}]
PN	Accumulated plant nitrogen (the amount *N* taken up by the plant, roots excluded) [g N $plant^{-1}$ or kg N ha^{-1}]
PNC	Plant nitrogen concentration [Percent of DM]
PNC_a	Plant nitrogen concentration on an area basis (PN/LA) [g N m^{-2}]
PNR	Average rate of daily accumulated plant N during the linear growth phase (PN/DAT) [kg N ha^{-1} day^{-1}]
RGR	Relative growth rate [day^{-1}]
SLA	Specific leaf area (LA/LW) [g m^{-2}]
W	Weight per unit ground area of plant dry matter exclusive of roots [g $plant^{-1}$ or t ha^{-1}]
Index $_c$	critical
Index $_{org}$	organic
Index $_m$	metabolic compartment
Index $_s$	structural compartment

Introduction

There has been a considerable research effort on developing techniques in soil and plant analysis to improve the efficiency of fertilizer use for a minimised environmental impact. The adoption of soil or plant analysis in vegetable production has however often been low (Hartz, 2004). The reasons for limited routine use of soil or plant analysis for appropriate plant nutrient management may be very different, for example time-consuming sampling or difficulties in establishing reference data against which the actual nutrient status of a crop can be evaluated. In this thesis reference data (critical concentrations) have been derived on the basis of a theoretical framework presented in the literature (Caloin & Yu, 1982: Caloin & Yu, 1984: Grindlay, 1997; Lemaire & Gastal, 1997; Lemaire *et al.*, 1997). First, the problems and possibilities hitherto encountered with soil and plant analysis for plant nutrient management, are reviewed. The review concludes with a suggestion for how some of the problems with plant analysis may be overcome with leaf area based plant analysis and by referring critical concentrations to biomass instead of time or development stage. The results are presented both on a basis of the whole plant and of individual leaves. Plant analysis is mainly diagnostic, as it only retrospectively reflects the plant nitrogen status, but may be a valuable tool for evaluation of plant nutrient management strategies attempting to more closely adjust supply to crop demand. The results are based on experiments conducted both under field conditions and in a climate chamber with the white cabbage cultivar SW Heckla (*Brassica oleracea* L. var. *capitata* L. f. *alba* D.C.).

Background

Soil and plant analysis for plant nutrient management – problems and possibilities

The ability to identify deficiencies or excesses of plant nutrients in crops is important in order to achieve an efficient utilisation of available nutrient resources for crop production. Excess nutrients in the soil-crop system will increase the risk of nutrient losses to the environment and the risk of impaired product quality (Belec *et al.*, 2001; Santamaria, 2006), whereas deficiencies will reduce crop production. The risk for the former is higher when high application rates are used, as is the case for many vegetable crops which have high nitrogen (N) demands and leave N rich crop residues after harvest (Goulding, 2000; Neeteson & Carton, 2001). This can appear to be a minor problem because the amounts of fertilizer used for vegetables are marginal in comparison to the total amounts used in agriculture, since the land area used is small. In Sweden, vegetables were grown on 7047 ha in 2005 (SCB and SJV, 2006a) of the total 2.7 million ha arable land (SCB and SJV, 2006b), while cabbage was grown on 370 ha (SCB and SJV, 2006c) compared to 113 590 ha in total in Europe (Eurostat, 2007; EU27; Portugal, Spain and UK not included). However, although the area is small and amounts of fertilizer used in vegetable growing are low compared to the total

amounts used, local effects on the environment can be considerable. Nitrate leaching may result in groundwater concentrations that exceed the emission limits of the EU Nitrate Directive (Monteny, 2001). The estimated average potential nitrate leaching in Denmark 1987-92 was twice as much on vegetable farms (122 kg N ha^{-1}) compared to farms growing standard arable crops (52 kg N ha^{-1}) (Huus-Bruun, 1993). Fertilizer strategies based on conventional recommendations and measures have been shown to be insufficient to meet the EU Nitrate Directive (Salomez *et al.,* 2005; van Dijk & Smit, 2006). Nutrient balances from different field vegetable production systems indicate large nutrient surpluses, suggesting that there is a potential to improve the efficiency in fertilizer use without reductions in yield (Neeteson *et al.,* 2003). The attitude of the farmers is important for progress, their motivation is necessary for a change in fertilizer strategy (Booij *et al.,* 2003). Evident indications of the nutrient status in their own fields and tools to measure and evaluate the adequacy of their fertilizer programs and nutrient management strategy may increase their motivation. This applies to both conventional and organic production. There is a wide range of tools for evaluation of the nutrient status of the soil or crop, ranging from simple tools that can be managed by the farmers themselves, such as the greenness in a "fertilizer window" (Rimpau, 1984), to methods of soil and plant analysis which require laboratory analysis.

Indicators of nutrient status

Whatever tool is used, its aim is to serve as an indicator of the actual nutrient status of the soil-crop system. Indicators can be used to evaluate the actual plant nutrient management strategy (diagnostic indicators) or to give predictive information such as information on the actual fertilizer requirement for the next application (prognostic indicators) (Lewis, 1993; Schröder *et al.,* 2000). The use of indicators to evaluate the actual practice implies a participatory learning process by which the farmer's motivation for a change is encouraged (Roling & Wagemakers, 2000). The management of plant nutrients can be successively improved by evaluation of the fertilizer strategy.

Generally, an ideal indicator must be reproducible (Schröder *et al.*, 2000). For evaluation of the nutrient status, the indicator should interpret the actual nutrient status of the soil-crop system in the same manner over different sites and years. The indicator can pertain to soil or to plant.

The soil mineral N as an indicator

Soil N analysis evaluates the soil mineral N supply in relation to the expected demand of the crop and serves as a prognostic tool as it predicts the fertilizer requirement for the remaining growth period. According to the N_{min}-method (Wehrman & Scharpf, 1979), the actual application rate of N fertilizer is estimated from the N demand of the crop and by adjusting for the actual soil mineral N content within the assumed rooting depth. A recommendation system, the KNS-system, compiles data on N demand for several vegetable crops (Lorenz *et al.*, 1989; Feller *et al.,* 2001). An important advantage with the method is that the

fertilizer requirement for any arbitrarily chosen period within the growth period can be estimated. A similar system has been developed in the US, the pre-side soil nitrate test (PSNT). Pre-side refers to seasonal applications beside the plants. The nitrate content of the soil is measured (Magdoff, 1991) as, for example, in the N_{min}-method. A pre-side nitrate test critical concentration is defined as the value above which no sidedress N is needed. Below the critical pre-side nitrate test concentration sidedress N should be applied according to a standard rate. Pre-side nitrate test critical concentration has been developed for different crops and also for vegetables such as cabbage (Heckman *et al.,* 2002). Considerable reductions in seasonal N applications without reduction in yield were made possible by use of the pre-side nitrate test in commercial celery and lettuce production (Hartz *et al*., 2000). According to Hartz (2003) the pre-sidedress soil nitrate testing is preferable to plant analysis for decision support for seasonal (sidedress) applications. Farmers have been encouraged to use the N_{min} method in vegetable production (Scharpf, 1991). However, adoption of the method for routine soil analysis by farmers has been low (Hartz, 2004). Sampling is time-consuming, especially below the top soil for deep-rooted crops. Acceptance by farmers has been better in areas where close co-operation with a nearby laboratory is possible. In such a co-operation, laboratory personnel were responsible for the soil sampling and provided recommendations immediately after sampling (Ziegler *et al.,* 1996).

Crop N status as an indicator

Plant analysis is an important tool for diagnostic evaluation of the nutrient status of a crop (Mills & Jones, 1996; Reuter & Robinson, 1997; Kalra, 1998). It has been widely used for identifying plant nutrition deficiencies and disturbances in crops but only to a lesser degree for routine evaluation of the plant nutrient status for adequate plant nutrient management.

An evaluation of the nutrient status is made possible only by relating the actual status to a standard. The concept of critical percentage, introduced by Macy (1936), can be used as such a standard or reference value. It suggests that there is a critical nutrient concentration for each nutrient and for each kind of plant. Lemaire & Gastal (1997) defined the critical N concentration as: at a given crop dry matter a certain critical plant N concentration (PNC_c) in the dry matter mass is needed to obtain the maximum instantaneous growth rate (GR). The PNC_c usually refers to optimal growth, but could as well refer to other properties such as susceptibility to physiological disorders or to diseases.

The results of plant analysis are affected by environmental factors such as soil and climate, and plant factors such as the plant's development stage which have to be taken into account when interpreting the result of the analysis (Lewis *et al.,* 1993). Also, critical concentrations may vary depending on the conditions when they were determined (Bates, 1971) and therefore critical nutrient ranges (CNR) have been preferred instead of a sharp limit between deficiency and sufficiency (Dow & Roberts, 1982). The use of CNR does however not allow for a precise determination of the nutrient status. Therefore, one of the reasons for the low adoption of plant analysis may be the difficulty in interpreting the results against

reliable reference or standard data because of the different factors affecting them. These factors more or less generally affect the outcome of plant analysis independently of the method used.

Several environmental factors may affect the results of plant analysis. The site affects the results of the analysis by various factors such as soil type (Westerveld *et al.,* 2003b), soil moisture content (Swaider *et al.,* 1988), fertilizer source (Barker *et al.,* 1971) and climate (Sorensen *et al.* 2006). Differences and variability in climate can be accounted for by relating growth to degree days rather than to time or growth stage (Grevsen, 1998). Plant nitrate concentration has been found to vary with the time of day. Lower nitrate concentrations are found in early afternoon and on sunny days (Iversen *et al.,* 1985). Scaife & Stevens (1983) recommended taking samples within two hours of midday.

Despite local variations in soil types and management, there was good agreement between critical petiole nitrate concentrations of potato derived from different studies by Gardner & Jones (1975), MacMurdo *et al.,* (1988), Porter & Sisson (1991) and Bélanger *et al.,* (2003). The three latter studies were conducted in the same region of Atlantic North East America, whereas the study by Gardner & Jones (1975) was conducted in Idaho in the North West. The same cultivars were used except by MacMurdo *et al.,* (1988). In spite of the variability in environmental conditions, the results support the possibility of establishing a general applicable critical concentration in regions with similar climatic conditions, as suggested by Bélanger *et al.,* (2003).

Nutrient interactions may affect the concentration of a certain element. Higher concentrations may appear when growth is limited by another element, compared to when the nutrients are available in adequate proportions. Focus is often on N being the most decisive element for growth. However, there is always the risk for erroneous interpretation of the results if the concentrations of other elements are unknown. Multi-element analysis is therefore preferable although more costly.

The critical petiole NO_3-N concentration and total N concentration vary with the cultivar as was shown for potato by MacMurdo *et al.,* (1988) and Porter & Sisson (1991) and for onion by Westerveld *et al.,* (2003b). Therefore, cultivar specific critical N concentrations may be needed.

Nutrient concentrations decline ontogenetically during the growth period, even with sufficient N supply (Siman 1974; Sorensen, 2000). Therefore, the critical concentration has to be related to a carefully defined growth stage (Lorenz & Tyler, 1977). However, the way the growth stages are defined is often imprecise. Typical examples of growth stages for cabbage referred to in the literature are “2 to 3 months old” (Mills & Jones, 1996) and “at heading” (Maynard & Hochmut, 1997). Westerveld (2003b) concluded that the main difficulty in using critical concentrations is to match the stage of sampling to the growth stages for which critical concentrations are given in the literature.

Concentrations vary between plant parts. The plant part to be sampled should be sensitive to variation in nutrient supply and should be easy to identify for correct sampling. For small plants whole shoots may be sampled, whereas for bigger plants sampling just a part of the plant is more convenient. The leaf is the plant part commonly recommended for sampling (Benton Jones Jr, 1985). According to Geraldson *et al*. (1973), the youngest fully mature leaf is preferable for nutrient analysis for many crops. Tabor *et al.* (1984) suggested sampling of fully mature leaves because immature and not fully extended leaves were less sensitive to changes in nitrate N content and the nitrate content varied considerably between immature leaves, whereas there was no significant difference in nitrate N content between the first, second and third mature leaf. For cabbage the wrapper leaves (Westerveld *et al.,* 2003b), the most recently fully expanded leaf and the youngest fully opened leaf which later become the wrapper leaves (Huett & Rose, 1989) have been sampled. Concentrations of nutrients vary between individual leaves at different positions (Dole & Wilkins, 1991). Therefore, the leaf position sampled for diagnostic analysis must agree with the leaf position that the critical concentration refers to. The lowest concentration is found in the upper leaves, except for the youngest leaves, as shown by Geyer & Marschner (1990) for maize. In contrast, leaves at the bottom of the canopy will first indicate a sudden deficiency as nutrients will be translocated from lower leaves to upper leaves in the case of deficiency (Girardin *et al.,* 1985; Ogunlela *et al.,* 1990). The variability is however larger for the lower leaves (Scaife & Stevens, 1983). Therefore, the lower leaves are not commonly used for plant analysis. Thus, there has been a general preference for choosing upper leaves for plant analysis, but it is difficult to define a very precise specific leaf position to sample. This may result in a certain variability in the analysis as concentrations vary between individual leaves.

Besides leaves, petioles are used for plant analysis. Petioles have often been used for quick tests to estimate nitrate-N in sap (tissue nitrate-N). The quick tests have been developed for field use to avoid the time lag between sampling and result as well as the costs of laboratory analysis. Petiole plus midrib nitrate has been shown to reflect the N status, as for example in potato (Bélanger *et al.,* 2003). The N status was based on measurements of total N and expressed by the nitrogen nutrition index (NNI) (Lemaire & Gastal, 1997). The NNI is defined as the ratio between the actual and the critical concentrations. The relationship between nitrate and NNI indicated a certain variability ($0.29 < R^2 < 0.62$). Petiole critical nitrate-N concentrations on a dry matter basis have been derived for several crops and also for brassicae such as broccoli (Gardner & Roth 1989a), cabbage (Gardner & Roth 1989b) and cauliflower (Gardner & Roth 1990). Linear relationships between field determinations of nitrate-N in sap and on dry matter in the laboratory have been found in many studies (Kubota *et al.,* 1996; Kubota *et al.,* 1997, Coulombe *et al.*, 1999). The quick tests provide rapid answers but with some loss in accuracy.

Westerveld *et al.* (2003b) estimated both nitrate-N and total-N but found it difficult to match concentrations from their experiments with recommended critical concentrations. Cabbage, carrot and onion with varying N status ranging from deficiency to excess were grown for two years and on two soils and the

tissue concentrations were compared with corresponding literature data on critical concentrations. They found that the nitrate concentrations were very variable so that total N matched literature data better compared to nitrate, however fertilizer rates according to these data would have resulted in either under or over-application of fertilizer. The main difficulty was the discrepancies and lack of accurate definitions of the stages of sampling. They concluded that a greater standardisation of sampling procedures would improve the usefulness of tissue analysis. High variability from tissue analysis was supported by Matthäus & Gysi (2001). Broccoli petiole sap nitrate-N varied between less than 2500 and up to a peak of 4000 ppm within a period of four days. Neverthelesss, the coefficient of variation was lower for the plant sap analysis (9%) compared to the soil-N_{min} analyses (29%). Moreover, the time required for sampling in the field was much lower for sampling petioles compared to extracting soil cores. Thus, plant analysis has advantages over soil analysis because there is less variability and sampling is less time consuming, however difficulties due to variability and defining the growth stage for sampling remain.

The predictive value of plant analysis has been under debate. For a predictive function, the nutrient status at a certain growth stage during the growing period should relate to final yield or to the optimum N application rate. Such relationships have been shown; for example yield of broccoli was related to nitrate in the midrib (press sap as well as on a dry matter basis) and to total N in the most recently fully expanded leaf at different growth stages throughout the growth period (Castellanos *et al.,* 2001). The optimum N rate for maize was related to tissue N and chlorophyll meter readings at the time of sidedress application (Scharf, 2001). However, such relationships are not generally found. A poor relationship between nitrate sap tests and N uptake of potato together with spatial and temporal variability caused MacKerron *et al.* (1995) to question the benefit of the sap nitrate test for adjusting top-dressings of N-fertilizers. Their criticism was directed against the prognostic value of the sap nitrate test. Neither was a relationship of sap nitrate to the optimum amount of N for top dressing found for Brussels sprouts in 46 field trials conducted by Scaife & Turner (1987). They concluded that the variations in the amount mineralised after top-dressing and in the N demand obscure such a relationship. Therefore, Scaife (1988) rejected attempts to derive critical concentrations of petiole sap nitrate from final yields but instead stressed the importance of relating the concentrations to the instant growth rate. Hartz (2003) concludes that plant analysis does not generate data that are useful for estimating appropriate seasonal N applications, but is valuable to identify N deficiencies. Thus, in spite of problems with variability, plant analysis may be useful for its diagnostic function but not for a predictive, prognostic function.

Leaf area - nitrogen - mass relationships; –whole plants

An important advance in taking account of nutrient variability was made with the observation that despite large differences in growth and nitrogen uptake rates of tall fescue (*Festuca arundinacea* Schreb. cv. Ludelle) between years, the relationship between the plant N concentration on a dry matter basis (PNC) and

the weight per unit ground area of plant dry matter (W) was the same in all years for plants grown under non N-limited conditions (Lemaire and Salette, 1984). This means that differences in climatic conditions between years affected crop growth and N uptake, but not the relationship between PNC and W. This overcomes the problems of variability caused by climate. The PNC has been found to decline in a typical pattern with an increase in the amount of biomass per unit ground area. The pattern can be described by the following equation:

$$PNC = aW^{b} \qquad (1)$$

where a (%) represents the initial N concentration at low biomass densities (< 1 t ha^{-1}), and b describes the pattern of the decrease of PNC with growth (Lemaire & Salette, 1984). For a non-limiting N supply, the parameter a was 4.8% and parameter b was –0.324 for tall fescue up to W around 6 t ha^{-1} (Lemaire & Salette, 1984). Moreover, this equation has been found to hold for a very wide variety of crops such as grasses, vegetable crops and cereals with the main differentiation being between plants with the C3 and those with the C4 pathway of photosynthesis, which differ in terms of parameter a rather than b (Greenwood *et al*., 1990). Therefore, species- or cultivar-specific critical concentrations may not have to be defined. Another advance is that the growth stage is continuously defined so that there is a unique PNC for each value of W. The problem in plant analysis of defining the growth stage or time for sampling is thereby avoided.

Mathematically different, but principally similar equations to describe the decline in PNC_c with plant biomass have been evaluated for a range of agricultural crops, including vegetables. Greenwood & Draycott (1989) used an exponential function for several vegetables, but a linear function specifically for brassicae (Greenwood *et al.,* 1996). The equations were derived from data sets for crops grown under non N-limiting conditions and do therefore not necessarily describe the relationship between PNC and W at optimum N supply (i.e. the lowest N supply and PNC that result in maximum growth) as some luxury consumption may have occurred (Greenwood *et al.,* 1986). To obtain the relationship between PNC and W at optimum N supply (PNC_c), data from multi-N level experiments are needed with frequent harvests during the growing season. Similarly, because of difficulties in statistically determining the PNC_c at each harvest with sufficient accuracy, data from a large number of experiments are also usually needed (e.g. Justes *et al.,* 1994 and Plénet & Lemaire, 1999). Riley & Guttormsen (1999) compared the equations describing the decline in PNC_c with W using data of PNC_c and W for cabbage obtained from multi-N level experiments in Norway. They found that the equation of Greenwood & Draycott (1989) underestimated PNC_c, whereas the equation proposed by (Greenwood *et al.,* 1996) overestimated PNC_c.

The equations mentioned above are entirely empirical and it is therefore not possible to test their validity experimentally. In contrast, Ingestad & Lund (1986) and Ingestad & Ågren (1992) developed theoretically well founded relationships between the relative growth rate (RGR) and PNC during exponential growth (that occurs in the plant's early growth phase) under conditions of steady-state with respect to the relative nutrient addition rate. Their studies have shown that

under these conditions, and with relative addition rate at optimal or suboptimal rates, PNC is linearly related to the RGR, and therefore, by multiplication by W, absolute growth rate to the amount of N in the plant. This therefore confirms the validity that under steady state conditions with a growth-limiting N supply, the resulting lower growth rate is reflected in a lower PNC of the plant. Even though the relation between RGR and PNC is well-established under conditions of constant relative addition rate and exponential growth, the relation between the declining PNC and increasing W after the period of exponential growth is not as well-founded in a theoretical framework.

After the exponential growth phase PNC and RGR decline ontogenetically (Greenwood *et al.,* 1991). During vegetative growth the cause of the decline in PNC is mainly attributed to a relative increase in 'structural' (supportive) tissues rich in cellulose and lignin with a low N content compared to the higher N content of 'metabolic' tissues accommodating the photosynthetic function with biomass production (Warren Wilson, 1972). Based on a conceptual model of the two compartments of plant nitrogen, Caloin & Yu (1984) related the decline in PNC to RGR. They assumed that the N concentrations of the two compartments PNC_m (metabolic) and PNC_s (structural) were constant and that the change in biomass proportion of the two types of tissues caused the decline in PNC. Moreover, they assumed that the GR was proportional to the amount of metabolic biomass by the proportionality constant k as this tissue accommodates the function for biomass production with photosynthesis. Based on this assumption, Caloin & Yu (1984) related PNC to RGR as

$$\mathrm{PNC} = (\mathrm{PNC}_m - \mathrm{PNC}_s)/k \times \mathrm{RGR} + \mathrm{PNC}_s \quad (2)$$

Equation 2 implies proportionality between PNC and RGR, but to examine the ontogenetic decline in relation to growth and development they have to be related to W. Caloin & Yu (1982) derived an expression for GR as a function of W:

$\mathrm{GR} = k\ \mathrm{W}^{\alpha}$

and in consequence

$\mathrm{RGR} = \mathrm{GR}/\mathrm{W} = k\ \mathrm{W}^{\alpha-1}$

where k is a constant and α a scaling exponent.
According to Caloin & Yu (1984), GR is proportional to the biomass of the metabolic compartment (W_m):

$\mathrm{GR} = k'\ \mathrm{W}_m$

This means a proportional relationship between W_m and W^{α} and a parallel decline of PNC and RGR at a rate of α–1. According to these theories, PNC can be predicted from RGR (Greenwood *et al.,* 1991).

Proportionality between PNC and RGR implies that PNC will decline parallel to RGR in relation to W according to the power function based on the theories of Caloin & Yu (1982) and Caloin & Yu (1984). It has been shown that the GR of many organisms, plants as well as animals, relates to W according to the 3/4 –

Power Law (Niklas, 1994), i.e. the scaling exponent α = 0.75. A similar relationship exists between surface area and biomass of cells and organisms according to the 2/3-Power Law (Niklas, 1994). Hardwick (1987) derived a 2/3-Power relationship for plant communities –the "core-skin" hypothesis. The hypothesis is based on the same idea of two compartments as described by Caloin & Yu (1984) with a "skin" of outer tissues engaged in energy exchange with the environment and a "core" of structural and supportive inner tissues. Based on the geometric relation between volume and periphery of a three-dimensional core relative to its length, Hardwick (1987) derives a 2/3-power relationship between "skin" and "core" and assumes that the amount of energetically active "skin" tissue is allometrically proportional to the accumulated plant N (PN). The 2/3 power relationship is in agreement with the value of the scaling exponent (0.63) in the relation between PN and W by Plénet & Lemaire (1999):

$PN = 34W^{0.63}$

Leaf area (LA) can also be assumed to be proportional to "skin" tissue which is supported by the similar allometric scaling exponent *b* (equation 1) found by Plénet & Lemaire (1999) for the leaf area index (LAI) as for PN:

$LAI = 1.234W^{0.679}$

PN and LAI therefore scale with similar proportionality to W with the allometric scaling exponent *b* making it possible to compare the rates of change between variables that have different units (Niklas, 2006). A similar scaling exponent b, therefore implies that LAI and PN will change at similar rates relative to W.

The similar values of the scaling exponents *b* in their relation to W indicates proportionality between LA and PN with a constant PN on an area basis (PNC_a). PN is linearly related to LA but not to W (Grindlay *et al.,* 1997). A linear relationship between PN and LA has been shown for various crops such as wheat (Sylvester-Bradley, 1990 and Olesen *et al*., 2002), tomatoes (Tei *et al*., 2002), Brussels sprouts and leeks (Booij *et al*., 1996) and cabbage (Ekbladh *et al*., 2007). Thus, the 1:1 relationship of LA and PN, during the exponential growth phase (Glimskär & Ericsson, 1999), continues also after the exponential growth phase, but not a 1:1 relationship of LA or PN to W.

Plant nitrogen is determined from, and therefore more conveniently expressed as the plant nitrogen concentration. The relationship between PN (kg N ha^{-1}) and W (ton DM ha^{-1}), $PN = 34W^{0.63}$ (Plénet & Lemaire, 1999) can be converted to PNC (equation 1) as

$PNC = 34/10 \times W^{0.63-1}$
$PNC = 3.4W^{-0.37}$

An expression for leaf area ratio (LAR) is obtained by a similar conversion of the expression of LA as a function of W ($b_{LAR} = b_{LA} - 1$).

The value of parameter *a* in equation (1), 3.4% for maize, represents the PNC of young plants in their exponential growth phase when most of the above ground plant biomass consists of photosynthetic tissue.

The value of parameter *b* found for maize (−0.37) is similar to that found for tall fescue by Lemaire & Salette (1984). The relationship for tall fescue was originally introduced as an empirical relationship but it can be linked through parameter *b* to the value of 2/3 suggested by the "core-skin" hypothesis. The allometric relationship between LA and PN implies a corresponding allometric relationship between LAR and PNC as $b_{LAR} = b_{LA} - 1$ and $b_{PNC} = b_{PN} - 1$ so there is a similar proportionality between LAR and PNC as between LA and PN. However as described above, Caloin & Yu (1982), Caloin & Yu (1984) and Greenwood *et al.* (1991) suggested proportionality between PNC and RGR. Proportionality implies similar rates of decline in both LAR and RGR relative to PNC. Similar rates would imply a constant net assimilation rate (NAR) as RGR can be factorised into its growth components LAR and NAR (Hunt, 1978). Moreover, in the case of a constant PNC_a, a constant NAR implies constant nitrogen productivity (NP = GR/PN) because NAR is $PNC_a \times NP$. As mentioned above PNC and RGR are linearly related with a constant slope (=NP) during the exponential growth phase (Ingestad & Ågren, 1992). After the exponential growth phase, nitrogen productivity was however expected to be reduced for example by self shading (Ingestad & Ågren, 1992). So, LAR, NP and RGR will change with growth, and the relative importance of their ontogenetic changes in relation to the ontogenetic changes in PNC needs to be determined in the attempt to relate the ontogenetic decline of PNC to any of them. It is the leaf nitrogen that is involved in biomass production, so to distinguish between the productivity of the leaf nitrogen and nitrogen allocation, nitrogen productivity can be factorised into leaf nitrogen productivity (LNP = GR/LN; LN=leaf nitrogen) and leaf nitrogen ratio (LNR = LN/PN) (Hirose, 1988). Then RGR can be factorised into:

$$RGR = LNP \times LNR \times PNC \qquad (3)$$

Moreover, NAR can be factorised into leaf nitrogen productivity and leaf nitrogen concentration on an area basis (LNC_a = LN/LA). The ontogenetic changes in LNC_a are of importance both for the relation of PNC to RGR and for plant analysis of individual leaves as proposed by Lemaire *et al.* (1997).

Leaf area - nitrogen - mass relationships – individual leaves

Sampling whole plants is inconvenient in the case of crops with large plants such as cabbage. Moreover, although the critical PNC can be well-defined in relation to W, the problem of estimating W for samples from commercial fields remains. As an alternative to sampling on whole plants, leaf area based assessment of leaf N content can be used instead as proposed by Lemaire *et al.* (1997). LNC_a has been shown to remain constant during growth and development of the last visible collar leaf of maize (Lemaire *et al.,* 1997) and for new leaves appearing on top of the canopy of lucerne (Lemaire *et al.,* 1991). The constancy of LNC_a of the top leaves offers a possibility of overcoming the problem in plant analysis of a varying N content according to the growth stage.

LNC_a of the top leaves remains constant because they are exposed to a constant light intensity and their LNC_a is adjusted to the ambient light intensity (Grindlay,

1997). The successively emerging new leaves at the top of the canopy are exposed to full light, to which the LNC_a for these leaves will be adjusted (Lemaire *et al.,* 1997). The unshaded leaves at the top of the canopy would be relatively easy to identify and to reach for sampling. For the sampling, an appropriate technique and tool is needed to punch out leaf discs of a well-defined leaf area. Alternatively, if the readings of chlorophyll meters can be calibrated to LNC_a, instant determination would be possible (Richardson *et al.,* 2002); however uncertainty remains about the constancy of the calibration equation over time. Good correlation, although not entirely consistent between years, between Minolta SPAD-502 chlorophyll meter readings and both tissue nitrate and total N found by Westerveld *et al.* (2003a) shows that the chlorophyll meter can be used for cabbage with good results.

Possibilities for plant analysis based on critical N concentrations derived from leaf area or growth

Plant analysis is mainly diagnostic as it retrospectively reflects the nutrient status. It is a powerful tool for evaluation of fertilizer strategies and may serve as a complement to prognostic methods with a predictive function. Farmers in vegetable production are encouraged to use the N_{min}-method or the pre-side nitrate test-test for adjusting the N application rates to the soil mineral N supply. Adoption by farmers has however been low and therefore research and development on computer-based recommendation systems are in progress. Eurotate-N, based on WELL-N and N_ABLE (Rahn *et al.,* 1996; Greenwood, 2001), and N-Expert (Fink & Scharpf, 1993; Fink & Feller, 1997) are the most important for vegetable production. Evaluation of WELL_N showed promising results as the combination of acceptable yields and low soil mineral N residues was achieved (Goodlass et al., 1997). These methods have been developed to predict fertilizer requirements by a precise matching of supply to demand. Trying to be precise may imply a certain risk for underfertilising compared to if fertiliser is applied with a certain margin of excess (Ekbladh, 2003) and therefore evaluation of the fertiliser strategy may be needed. Thus, the diagnostic function of plant analysis provides a complement and can be used together with prognostic methods. Organic vegetable production is another example where evaluation of the nutrient management strategy is of great importance. The strategy is often a combination of several practices, such as crop rotation and use of organic fertilizers for each of which the nutrient effect is difficult to predict. Plant analysis may serve as an indicator from which the management techniques can successively be improved in a participatory learning process, in which both farmers and advisers are engaged.

Plant analysis may advance by deriving critical concentrations according to the principles outlined above, so as to overcome some of the problems and drawbacks stated above:

- Growth stage: The most important advantage expected is that a continuously defined N concentration with growth eliminates the difficulty in defining specific growth stages to which the sampling date have to match. This applies both to analysis of the whole plant and of individual leaves.

- Plant part: According to the theory of the relationship between LNC_a and light intensity, LNC_a remains constant for unshaded leaves on top of the canopy. Sampling of top leaves is in agreement with earlier praxis.
- Differences between crops and species: The theories refer basically to general principles for the relation between growth and N; crop specific deviations from the theories need to be further explored.
- Environmental variability: Relating N concentration to growth instead of to time removed much of the variability between years. Moreover agreement has been found between critical concentrations determined within regions with similar climate and favourable growth conditions.
- Nutrient interactions: Nutrient interactions occur independently of method used. However, in contrast to soil analysis, multi-element analysis offers the possibility of showing the nutrient composition experienced by the plant.
- Total N of leaves gives less variability compared to petiole nitrate.

Aims and hypothesis

The overall aim of this work was to advance plant analysis as a tool for evaluation of the crop N status by establishing critical N concentrations based on a) the relationship between plant nitrogen concentration (PNC) and weight per unit ground area of plant dry matter (W) for whole plants, and b) a constant leaf nitrogen on an area basis (LNC_a) for leaves exposed to constant light intensity for analysis of individual leaves. The work builds on the theoretical frameworks proposed in the literature that describe the relation between N and growth.
Specifically the study was intended to

I: compare the rate of ontogenetic decline in PNC with the rates of decline in relative growth rate (RGR) and the growth components of RGR (leaf area ratio (LAR), leaf nitrogen productivity (LNP), leaf nitrogen ratio (LNR) and net assimilation rate (NAR) with the aim of finding a predictor of the rate of decline in PNC;
II: estimate the PNC_c for white cabbage, including the pre-linear growth phases.
III: relate the rate of decline in the derived PNC_c to the rate of decline in LAR at ample N supply;
IV: examine if a constant leaf area based value of the critical N concentration can be used for unshaded leaves at top of the canopy.

The main hypotheses of this study were that during growth and development, LNC_a of unshaded leaves at top of the canopy as well as whole plant PNC_a remain constant and that as a consequence of a constant PNC_a, LAR and PNC will decline at similar rates relative to W. An alternative hypothesis was that PNC declines at a similar rate as RGR.

Materials and Methods

The relationships between N and growth components of white cabbage were examined in both field experiments (Paper I and IV) and in a climate chamber (Paper II and III). The time-course of change in N concentrations and in growth components was examined by repeated samplings from seedling to mature plant during the entire growth cycle, both for individual leaves and for the whole plant. Data from the field experiments were used to describe the ontogenetic decline in PNC_c and to relate it to LAR under ample N-supply. Experiments were conducted in climate chamber, to closely examine the relation between growth and N without interference from fluctuations in N supply or in other environmental conditions.

Ontogenetic changes were compared by means of scaling relationships between various growth components and variables derived from the measured variables of leaf area, N and weight. Variables over different scales can be related by scaling relationships (Wright & Westoby, 2001; Niklas, 2006). The scaling exponent *b* in the power function $y=ax^b$ or the scaling coefficient b in the linear regression $\log(y) = \log(a) + b \times \log(x)$ show the proportional relationship between changes in the variables x and y, and where x and y can be of different units (scales) as in allometric relationships. The studied variables (y) were all related to W, i.e. (x) and the rate of their ontogenetic changes relative to W were described by the coefficient *b*. The variables compared were leaf area (LA) and accumulated plant nitrogen (PN) and the growth components leaf area ratio (LAR), leaf nitrogen on an area basis (LNC_a), leaf nitrogen productivity (LNP), leaf nitrogen ratio (LNR), net assimilation rate (NAR), plant nitrogen concentration (PNC) and relative growth rate (RGR). A similar rate of decline in PNC and another variable indicated a relationship although necessarily not a causal one. In addition, for direct comparisons with PNC, the scaling relationships were used to show how the data fit to the theories.

The relationship between PNC and growth components during the exponential growth phase was studied on plants grown in a spray-based flowing solution system (Ingestad & Lund, 1986) by which nutrient additions were automatised. Nutrients were added to an extent just compensating for nutrients removed from the culture solution. Nutrients were kept at a very low concentration and were replenished by small nutrient additions at short intervals, thus minimising “luxury” consumption. A major advantage of this technique is that the plant regulates its own nutrient demand and it avoids effects of fluctuating N concentrations in the plants caused by an N supply rate that does not closely match N demand, as occurs in the field or in traditional nutrient film or pot-growing techniques. This technique of growing plants at optimum conditions offers the possibility of carefully examining relations of PNC to W within the theoretical framework described above.

The growth device could only accommodate very small plants (exponential phase). For the growth phases thereafter, the principle of growing plants in large volumes of culture solution whereby the nutrient concentration of the solution

could be maintained constant, as the uptake is negligible relative to the amounts available in the culture solution, was used (Asher *et al.,* 1965). In both of the systems, plant nutrients are available at free access. The ontogenetic changes are thereby controlled by the plant itself. The alternative would be to control nutrient availability by applied rates. Then, however, there would be the risk of imposing changes in nutrient status which could interfere with the ontogenetic changes.

The PNC_c was estimated from growth response curves for N supply ranging from limitation to excess. Separate response curves were used for the pre-linear and linear growth phases. For the pre-linear growth phase response functions (piecewise regression and inverse polynomial regression) of PNC against W were used. For the linear growth phase a response curve was used that related growth rate (GR) to the parameters a and b of equation 1. The critical N concentration was related to 95% of the maximal GR (Olfs, 2005). Details are given in Paper IV and in Figure 3 in Paper IV. For determination of critical N concentrations data from T2001, T2002a and T2002b were used.

In all experiments white cabbage (*Brassica oleracea* L. var. *capitata* L. f. *alba* D.C. cv. Heckla F1, Svalöf Weibull AB, Hammenhög, Sweden) was used. The field experiments were carried out in SLU, Ultuna Horticultural Research Station, Uppsala (N59°49', E17°39') (Paper I) and Torslunda Experimental Station (N56°38´, E16°31´) (Paper IV). The soil at Ultuna was a clay loam and at Torslunda a loamy sand. Irrigation was applied according to a deficit balance of precipitation and evapotranspiration. Between and in-row plant spacings were 0.6 and 0.5 m, respectively. Plant protection measures and weed control were carried out as needed. Full supply of nutrients other than N (P, K, Mg, S and micronutrients) was applied to all treatments (N-levels). In total, seven experiments were performed as follows:

Ultuna 2000 (U2000) (Paper I)

Multi-harvest (0, 7, 14, 22, 35, 49, 63, 77, 97 and 114 days after transplanting, DAT) and multi-N-level (0, 25, 50, 100, 150, 200 and 250 kg N ha^{-1}) field experiment. N was applied as $Ca(NO_3)_2$ with ⅛ applied at transplanting (0 DAT), ¼ 23 DAT, ⅜ 50 DAT and the remaining ¼ of the N applied 78 DAT. The experiment had a split-plot design with N rate in the main plots and harvest day in sub-plots with three replicates in complete blocks. Four plants were harvested from each sub-plot and combined to provide one sample from each treatment and block/replicate. Determination of leaf area, total N and dry weight.

Torslunda 2001 (T2001) (Paper IV)

Multi-harvest (0, 10, 18, 26, 38, 52, 67, 84, 101 and 130, DAT) and multi-N-level field experiment (0, 50, 100, 150, 225, 300 and 375 kg N ha^{-1}). Fertilizer N applications were split at 11, 15, 23, 23 and 28% of the total N rate and applied immediately after harvests at 0, 27, 53, 67 and 94 DAT. The treatments were arranged in a split-plot design with N rate in the main plots and harvest day in sub-plots with three complete replicate blocks. At each harvest day 8 plants were

harvested and combined to provide one sample from each treatment and block/replicate. Determination of total N and dry weight.

Ultuna 2001 (U2001) (not presented elsewhere)

Multi-harvest (7, 14, 21, 35, and 49 DAT) and multi-N-level pot experiment. Chlorophyll content meter readings (CCM200, ADC BioScientific Ltd, Hoddesdon, Herts, UK). Determination of leaf area, total N and dry weight. Data from this experiment were used to study how chlorophyll content meter readings vary for different N-application rates for different individual leaves at 14, 35 and 49 DAT and to study the effect of varying N supply rates on LNC_a at 49 DAT.

Torslunda (T2002a) (Paper IV)

Multi-N-level field experiment for the pre-linear growth phase. The experiment consisted of seven different rates of N supply with three replicate blocks and was harvested during the exponential growth phase (30 DAT). Number of plants sampled, and design of experiment were as described for experiment T2001. Fertigation was used for nutrient supply to ensure a rapid, direct transport of nutrients to the roots. Determination of total N and dry weight at 30 DAT.

Torslunda (T2002b) (Paper IV)

Multi-harvest (1, 20, 26, 33, 40, 47, 54, 61, 68, 75, 82, 96, 110 and 134 DAT) field experiment at only one application rate: ample N supply. The amount applied was determined from the expected N uptake and from weekly soil mineral N samplings and analyses. The weekly demand was calculated on basis of maintaining a buffer of 50 kg N ha^{-1} in the soil, the expected weekly uptake (based on data from experiment T2001) and the actual soil mineral N measured two days before the next application. At each sampling time 14 plants were harvested and combined to one sample from each of the two replicate blocks. Determination of leaf area, total N and dry weight.

The Biotron (B2004) (Paper II)

Multi-harvest (5.9, 10.4, 11.9, 13.9, 17.2, 20.9 and 24.0 DAT) experiment conducted during the exponential growth phase in growth units (Ingestad & Lund, 1986) in a climate chamber (the Biotron, Alnarp) with one level of nutrient supply at free access. Determination of leaf area of individual leaves, nitrate-N, total N and dry weight.

The Phytotron (Ph2004) (Paper II and III)

Multi-harvest (1, 21, 29, 36, 41, 54, 62, 76, 90 and 104 DAT) pot experiment during the post-exponential growth phases in a flowing solution system in a climate chamber (the Phytotron, Ultuna, Uppsala) with one level of nutrient supply at free access. Determination of leaf area of individual leaves, chlorophyll, photon flux

density (PFD) distribution within the canopy, neutral detergent fibre (NDF) nitrate-N, total N and dry weight.

Results and discussion

Growth – determination of growth rate and relative growth rate

Growth analysis (Hunt, 1978) was the major tool used to relate N to growth by the means of growth components. GR and RGR were used in the growth analysis and were estimated by linear or non linear regressions of W (or lnW) against DAT. Growth was characterised by four growth phases. Growth was exponential (lnW to DAT, $R^2 = 0.999$) for the plants grown in the growth device of Ingestad & Lund (1986) (Figure 1). W increased from 4.6 mg of the seedling (germ) to 2 g per plant at 24 DAT. The next phase, the approximately exponential one, was characterised by a good non linear fit of W to DAT ($R^2 = 0.997$) but a gradual decline in the slope of the linear regression of lnW to DAT resulting in a poor fit of the linear regression ($R^2 = 0.93$) (Figure 1). During this phase, W increased to around 50 g. After a short transition phase, growth was linear in all experiments (Figure 1c and Figure 1 in Paper IV). The very last sampling date at all field sites was not included in the linear regression as GR declined. W at final harvest was slightly more than 600 g, similar for plants grown in the climate chamber and the maximal yield in the field (T2002b) and corresponded to 20.6 t ha^{-1}. The plants in the climate chamber grew faster (GR = 9.5 g $plant^{-1}$ day^{-1}; Figure 1) compared to those in the field (GR = 6.2 g $plant^{-1}$ day^{-1}; Figure 1 in Paper IV). The cabbage plant of cultivar Heckla developed around 35 green leaves, the leaves developed thereafter formed the head.

Whole plant critical N

The critical N concentration on a whole plant basis (PNC_c) for white cabbage cv. Heckla was estimated as (Paper IV):

$PNC_c = 4.5$ (% of DM) $W < 1.5$ t ha^{-1}

$PNC_c = 5.1W^{-0.33}$ (% of DM) $W > 1.5$ t ha^{-1} (eq. 1)

The PNC_c for $W < 1.5$ t ha^{-1} was estimated at 30 DAT. The value of 4.5% was calculated using an inverse polynomial, whereas piecewise regression estimated the value to 4.7 with the confidence interval of 4.60–4.74% (Figure 2 in Paper IV). The PNC_c for $W > 1.5$ t ha^{-1} was calculated from GR and parameters *a* and *b* of equation 1 during the linear growth phase and the confidence intervals were for a_c = 4.71–5.62 and for b_c = (–0.30) – (–0.36) based on the regressions of parameters *a* and *b* against the average rate of daily accumulated plant N (PNR) (Paper IV).

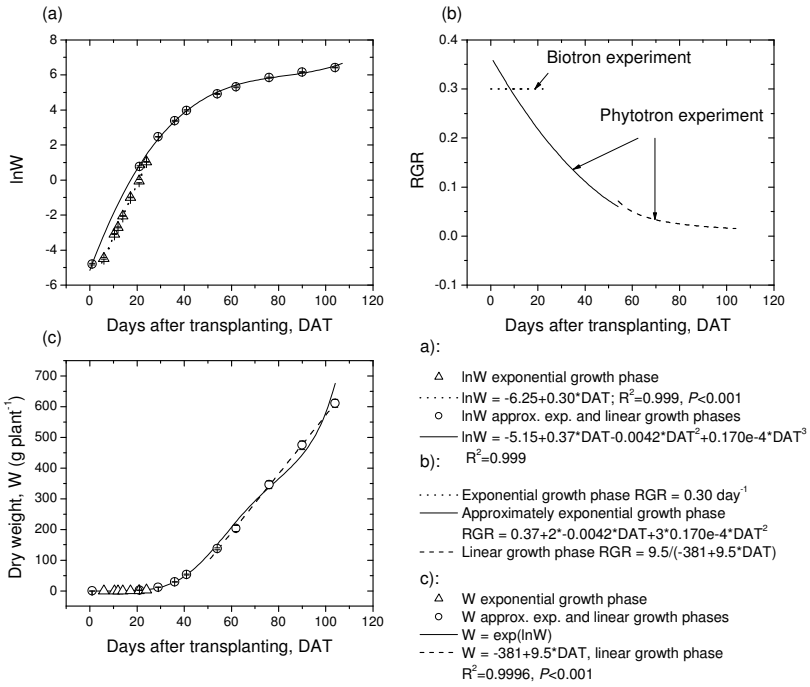

Figure 1. Growth and determination of RGR: a) lnW against DAT, b) RGR = d(lnW)/dt (slope of lnW) and c) growth curves: W = exp(lnW) (1-104 DAT) and W = a + GR × DAT (54-104 DAT). Exponential phase: Biotron experiment (up triangle); approximately exponential and linear growth phases: Phytotron experiment (circle). Error bars denote ±SE. (=Figure 1 in paper II).

The flat slope of parameter *a* against average rate of daily accumulated plant N (Figure 3b in Paper IV) implies an uncertainty in parameter a_c. The flat slope was most likely caused by the weak response to fertiliser N at the beginning of the growth period. Response of young plants to soil mineral N is not always to be expected (Binford, 1992). The recovery of fertiliser is low during early growth, especially for row-grown transplanted vegetables (Greenwood *et al.,* 1989). In this study when applied as a nutrient solution at a very wide range of N rates PNC of field grown young plants responded to different N supply rates (experiment T2002a, Paper IV).

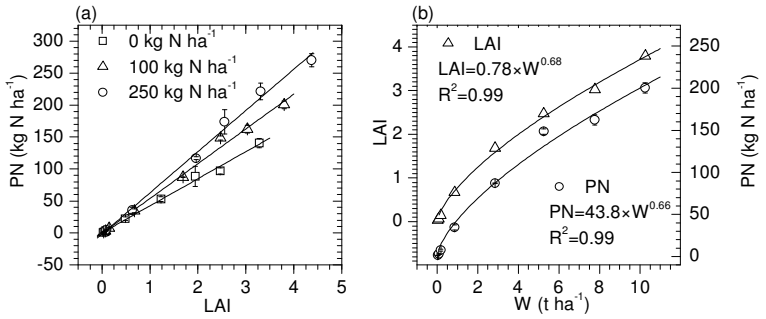

Figure 2. a) Plant nitrogen (PN) versus leaf area index (LAI). Data from N application rate 0 (PN = 42.3 × LAI + 0.27, R^2 = 0.99), 100 (PN = 54.4 × LAI – 0.36, R^2 = 0.99) and 250 kg N ha^{-1} (PN = 64.4 × LAI – 1.42, R^2 = 0.99) are shown for illustrative purpose; b) Leaf area index (LAI) and plant nitrogen (PN) versus dry weight of plant. Data from N supply rate 100 kg N ha^{-1} and DAT 1–97. Error bars indicate ±SE. Data from the U2000 experiment (=Figure 3 in Paper I).

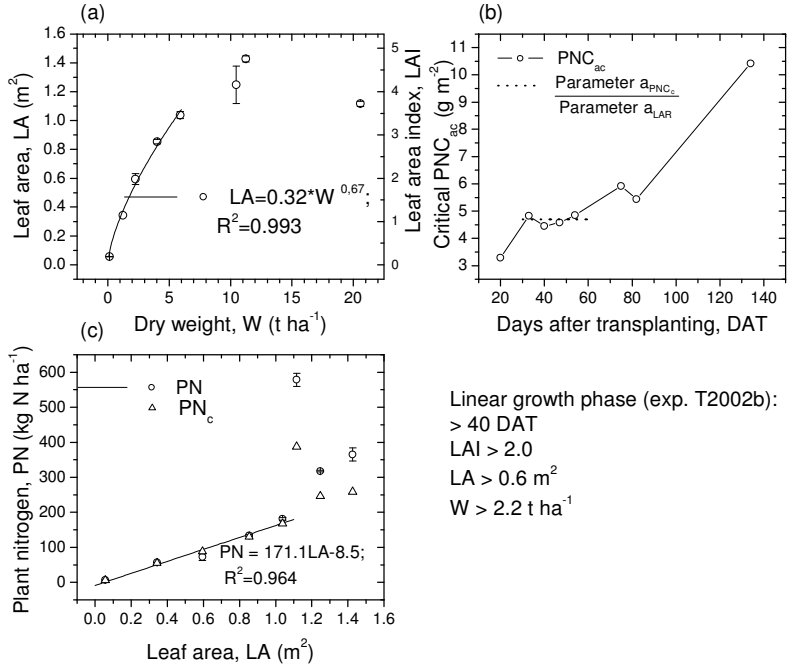

Figure 3. Relations to leaf area (LA) and leaf area index (LAI). a) LA against W; b) Critical plant nitrogen conc. on an area basis (PNC_{ac}), dotted line shows the ratio a_{PNCc}/a_{LAR}; c) Plant nitrogen PN and PN_c against LA. Measured data from experiment T2002b. Error bars denote ±SE. (=Figure 5 in Paper IV).

Plant N per unit leaf area (PNC_a)

The results from all experiments supported the main hypothesis of a constant PNC_a, except in cases where luxury consumption was suspected. PN was linearly related to LA in all of the three experiments where LA was measured (Figure 2; Figure 2a in Paper II; Figure 3), which is in agreement with linear relationship between PN and LA for various crops as described above. PNC_a remains constant as the rate of increase of mass with structural functions is made up for by the lower concentration of that mass (Grindlay *et al.,* 1997). To examine the time-course of PNC_a in more detail during growth and in order to relate PNC_a to changes in growth components, PNC_a was calculated for each sampling date (PNC_a = PN/LA). Under field conditions (U2000), PNC_a remained constant with growth, expect for the highest N supply rate (Figure 4). However, PNC_a for plants grown in the climate chamber increased (Figure 2b in Paper II), most likely because of luxury uptake of N. The critical PNC_a, PNC_{ac}, was calculated to be 4.7 g N m^{-2} (total N) by dividing parameter a_{PNCc} (5.1) by parameter a_{LAR} (0.011) (Figure 3).

Ontogenetic changes in PNC and in growth components

During the exponential growth phase, not only LA and PN, but also W increased at a close to 1:1 relationship to LA and PN, as shown for grasses by Glimskär & Ericsson (1999). The scaling coefficients for LA and PN relative to W were very close to 1 (Figure 3 in Paper II), which implies constant LAR, PNC and PNC_a. The

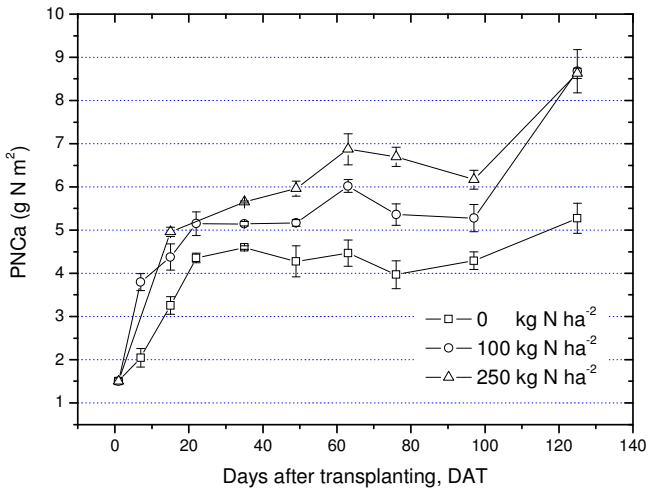

Figure 4. Accumulated plant N per unit leaf area (PNCa). Data from the experiment U2000. Error bars show ±SE.

constant RGR (Figure 1) implies a 1:1 relationship of GR to W, so that NAR would also have been constant. The constant relationship between PNC and RGR means a constant nitrogen productivity, which is the base for theory of N nutrition and growth proposed by Ingestad & Ågren (1992). Nevertheless, in spite of the 1:1 relationship between LA and W, the slope of decrease in LAR (linear regression of logLAR against logW, 10–24 DAT) was significantly different from zero (p=0.0094) (Figure 5a). Thus, LAR declined slightly in spite of the almost 1:1 relationship of LA to W. And as LAR is a growth component of RGR, the constant RGR during the exponential growth phase (0.30 day^{-1}) was most likely an approximation in spite of the very good fit of lnW to lnDAT (Figure 1a). Smolders (1991) also showed by means of accurate non-destructive measurements that RGR declines slightly during the exponential growth phase of spinach. During the first sampling interval in the Ph2004 experiment (21–29 DAT), LAR still declined at similar rate as during the exponential phase in the Biotron experiment (10–21

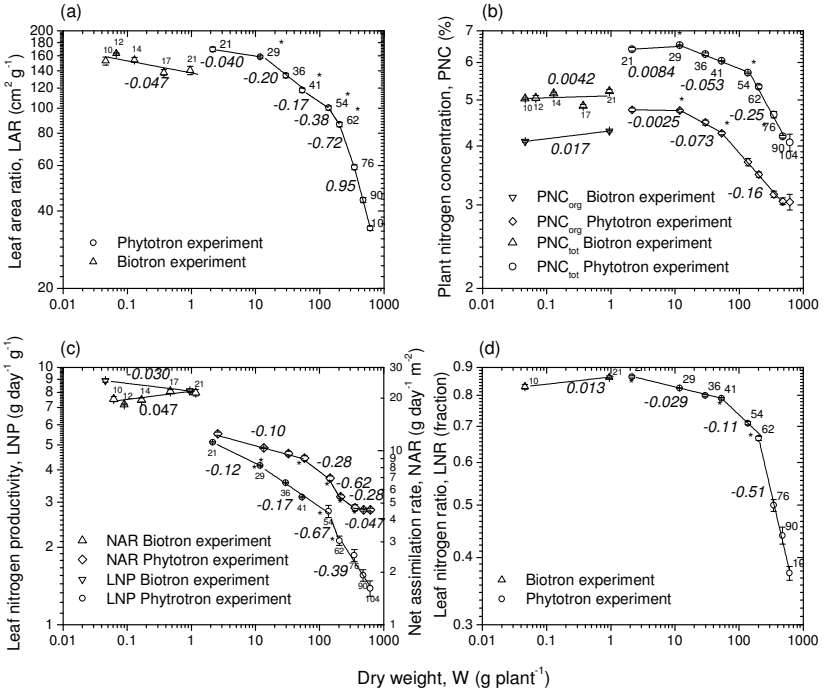

Figure 5. Time-course of change in a) leaf area ratio (LAR), b) organic and total plant nitrogen concentration (PNC_{org}, PNC_{tot}), c) leaf nitrogen productivity (LNP) and net assimilation rate (NAR) and d) leaf nitrogen ratio (LNR). The lines are the log-log linear regressions slopes and the figure in italics adjacent to the line indicates the slope (scaling coefficient, b_i, eq. 4 in Paper II). Smaller figures adjacent to data points denote days after transplanting (DAT). An asterisk at a data point indicates that the two adjacent slopes are statistically different ($P < 0.05$). Error bars denote ±SE. (Figure 4 in Paper II).

DAT) (Figure 5a). According to the hypothesis of a constant PNC_a, PNC can also be expected to decline at a similar rate as LAR during the exponential phase. However, in the Phytotron experiment PNC_a increased because of suspected luxury consumption and a decline in PNC, as in LAR, could not be confirmed by the data. PNC was almost constant during the exponential phase, in the Biotron experiment and up to 29 DAT in the Phytotron experiment (Figure 5b).

During the approximately exponential growth phase, there was no longer a 1:1 relationship of LA and PN to W (Figure 3 in Paper II), neither was there of GR to W as RGR declined (Figure 1b). PNC and RGR declined at different rates, –0.07 and –0.30 (Figures 5a and 5c). The rate of decline in RGR is the sum of the scaling coefficients of LAR (–0.20) and NAR (–0.10). Their different rate of decline means in terms of the theory by Ingestad & Ågren (1992) that nitrogen productivity declined. Leaf nitrogen productivity was responsible for the major part of the difference in rates of decline between PNC and RGR as leaf nitrogen ratio was nearly constant during the approximately exponential growth phase and in the beginning of the linear growth phase (Figure 5 in Paper I; Figure 5b, 5c and 5d). However, as shown for field data, the 1:1 relationship of PN to LA remained, as PNC_a was constant and LA and PN increased at similar rates relative to W (Figure 2b). Thus, the constant relationship between RGR and PNC during the exponential phase changes to a constant relationship between PNC and only one of the growth components of RGR, LAR. The difference between the rates of decline in PNC and RGR were due to changes in NAR and nitrogen productivity (NP). NAR and nitrogen productivity will change at similar rates as long as PNC_a is constant ($NAR = PNC_a \times NP$). The deviations between NAR and the sum of leaf nitrogen productivity and leaf nitrogen ratio shown for the rates of decline for the Phytotron data were related to the fact that PNC_a increased, contrary to what was hypothesised (Figure 4c and 4d in Paper II). For the same reason the rates of decline in LAR and PNC differed. The changing relationships between GR, LA, PN and W between the exponential and the approximately exponential growth phases were reflected in the change to a steeper rate of decline for LAR and PNC_{org} (Figure 5a and 5b). The scaling coefficient for LAR was −0.04 during the exponential phase and −0.20 during the approximately exponential phase. Thus, one level of rate of decline was associated with the exponential growth phase and another rate with the approximately exponential growth phase, rather than a gradually decreasing rate of decline. However, the critical N concentration for field-grown young plants was approximated by a constant value of 4.5% during the whole pre-linear growth period because the rate of decline for young plants could not be estimated under field conditions.

During the linear growth phase, PNC_a was constant in the field (Figure 4) so that LAR and PNC declined at similar rates, $b_{LAR} = -0.32$ and $b_{PNC} = -0.31$. These values were estimated from regressions of LA and PN against W for the entire growth period at U2000, 1–97 DAT (Figure 2b). They agreed with the value for critical PNC, $b_{PNCc} = -0.33$ (Paper IV) and for LAR at ample supply, $b_{LAR} = -0.33$. The latter value was estimated from the regression of LA against W up to 62 DAT as $b_{LA} - 1 = 0.67 - 1$, up to 62 DAT. After 62 DAT this relationship did not hold as leaf extension ceased. The rate of decline in PNC_c during the entire

linear growth phase could thus be predicted from b_{LA} up to 62 DAT. Also, in the Phytotron experiment PNC and LAR declined at similar rates ($b_{PNCorg} = -0.16$ and $b_{LA} - 1 = 0.85 - 1 = -0.15$) up to the time when the extension of LA ceased at 62 DAT (Figure 5b and Figure 3 in Paper II). Both experiments therefore supported the hypothesis of similar rates of decline for LAR and PNC. Direct regression on data of LAR and PNC against W gave more variable values of b_{LAR} and b_{PNC} and larger confidence intervals. Direct regression analysis was not possible for climate chamber data as only data from one sampling interval during the short period of the linear growth phase before heading were available. During heading LAR declined more sharply so that LAR and PNC declined at different rates. PN increased because of growth of the head whereas LA extension ceased. LAR seemed to gradually become more and more responsible for the decline in RGR and finally declined at a rate of −1 whereas the decline in NAR ceased. RGR declined at a rate of −1 because GR was constant: RGR = GR/W = GR * W^{-1}. Because of the increasing PNC_a, NAR and nitrogen productivity changed at different rates. NAR was constant because GR and LA were constant (Figure 5c), whereas leaf nitrogen productivity decreased because of increasing leaf thickness (Figure 5c) and leaf nitrogen ratio decreased mainly because of head growth (Figure 5d). Thus, none of the relationships shown during the vegetative phase before heading held during heading. Therefore, LAR and PNC can only be expected to be related during vegetative growth as long as leaf extension and mass develop proportionally and N is not allocated to other plant organs. In spite of this, PNC continued to decline at the same rate during the entire linear growth phase. In summary, the results therefore show that the rate of decline in PNC_c could be predicted from the rate of increase in LA with ample N supply.

The rate of increase in LA, $b_{LA} = 0.68$ across all N rates in U2000 (1–97 DAT) (Figure 2) and $b_{LA} = 0.67$ with ample N supply in T2002b (1–62 DAT) (Figure 3a) during the linear growth phase agree very well with the 2/3-Power Rule (Niklas, 1994) or "core-skin" hypothesis (Hardwick, 1987) which postulates that plants and plant communities that optimise the utilisation of the available radiation for growth will increase their PN in proportion to $W^{2/3}$ in a dense canopy. This applies to LA as well. LA and PN will increase allometrically at the same rate (the same scaling exponent) relative to W (Figure 3 in Paper I), because of the constant PNC_a. The "core-skin" hypothesis was forwarded to supplement the self-thinning rule, which states that the increase in mean mass per plant is related to a decrease in the number of plants in a dense plant community (Westoby, 1984). The energy available for growth is limited by the amount of light intercepted per ground area (Donald, 1961). When a plant dies, its share of energy flux can be used by neighbouring plants. Although the self-thinning rule only applies to dense canopies and not to row-grown crops such as cabbage, the growth of row-grown crops is limited as well by the incident light flux per unit ground area, which estimates the maximal daily growth rate that can be reached during the linear growth phase. The basic idea of the core-skin hypothesis, that periphery (LA and PN) relates to the length squared of a three dimensional core and the volume (proportional to W) to the length cubed, apparently also applies to cabbage although the leaves to a great extent were responsible for the increase in mass (Paper II), rather than the stem. When growth is limited by light, LA and PN in cabbage allometrically relate to W

by the proportionality factor 2/3, as periphery relates to volume, as proposed by Hardwick (1987).

The rates of decline in PNC were different in the climate chamber compared with those in the field. In the climate chamber, the average rate of decline in PNC_{org} during the linear growth phase was –0.16, which was similar to the rate of decline in LAR during the approximately exponential growth phase. This is in contrast to PNC of plants grown in a closed canopy (i.e. linear growth phase) in the field. For the latter, LAR and PNC declined in agreement with the core-skin hypothesis at a rate of –1/3. The rate of decline of LAR and PNC in the climate chamber was halfway between when LAR and PNC were close to constant (zero decline rate) and when they were –1/3 for the closed canopy, i. e. approximately –1/6. Therefore, results from the climate chamber cannot directly be applied to field conditions. The reason for the difference between the intermediate rate of decline and the rate of decline of –1/3 for cabbage plants grown in the climate chamber and cabbage grown in the field was mainly the difference in leaf size and thickness, with the former being larger and thinner. The mass fractions were similar for cabbage in the three experiments for which plant parts were weighed separately (U2000, T2002a, Ph2004, data not shown) so that the difference in rate of decline can only be attributed to the difference in specific leaf area (SLA). Broccoli grown at different plant densities by Francesangeli *et al.* (2006) behaved in a similar manner – mass fractions were unaffected whereas LAR increased at higher plant densities whereby the degree of shading increased. The larger LA of the cabbage in the climate chamber resulted in a lower rate of decline in LAR compared to the decline under field conditions and was most likely caused by the lower light intensity in the climate chamber and because the plants were grown as isolated plants. Rates intermediate between 2/3 and unity have also been shown,in other studies, such as for example 0.84 and 0.88 for PN $\propto$ W for lucerne grown in a greenhouse at low plant densities and at high densities under field conditions, respectively (Lemaire *et al.,* 2005). Values of 0.76–0.79 were found for the scaling exponent α of the relation between metabolic tissues and total biomass $W_m = k'(W)^{\alpha}$ proposed by Caloin & Yu (1982). These values were based on experiments with *Dactylis glomerata* in a climate chamber with light intensities of 35–85 W m^{-2}. The values for both of the experiments are closer to the 3/4-Power Rule by Niklas (1994) or to 5/6 instead of to the 2/3-Power Rule. It is evident that the allometric scaling exponent for LA and PN increases at a lower rate (2/3) within the constraints of a canopy compared to plants grown at low plant densities or at low light intensities. This applies to plants with very different morphology such as grasses and cabbage.

Effect of self shading on the ontogenetic decline in PNC

The increasing difference in rate of decline between PNC and RGR was, as shown above, attributed to decline in the nitrogen productivity and specifically in the leaf nitrogen productivity. After the closure of the canopy (the linear growth phase) GR was constant, which resulted in a decreased NAR as LA still increased at the very beginning of the linear growth phase. Leaf nitrogen productivity and NAR declined rapidly, with scaling coefficients of −0.62 and −0.67 respectively, compared to the

rate of decline of −0.16 in PNC_{org} (Figure 5b and 5c). Similar rates of decline were found for leaf nitrogen productivity and NAR under field conditions during the first part of the linear growth phase (Figure 5 in Paper I). The decline in leaf nitrogen productivity and NAR was preceded by a decline in the average amount of photon flux density (PFD) incident on the LA of the cabbage (Figure 5 in Paper II). NAR is known to be closely related to light intensity (Blackman & Wilson, 1951; McDonald *et al.,* 1992). The decline in leaf nitrogen productivity and NAR was therefore most likely caused by self shading. The hypothesis by Caloin & Yu (1984) related the ontogenetic decline in PNC to the ontogenetic decline in RGR which was based on an assumption that GR is proportional to the amount of metabolic tissue in the plant (W_m). The proportionality constant *k* was assumed to be constant for a given set of environmental conditions. But the conditions within the canopy change with increasing self shading. The proportionality constant *k* is not constant when self shading occurs and PNC and RGR are then not related, which confirms the reservation by Lemaire & Gastal (1997) of the constancy of constant *k* in a dense canopy. It is most likely that the metabolic tissue increases in proportion to the increasing leaf area. GR responds more directly to changes in light intensity than W_m. A relation of W_m to LA rather than to GR is therefore more likely and it can be shown that PNC relates to LAR rather than to RGR by changing the assumption of a constant GR/W_m to that of a constant W_m/LA (appendix B). The rapid response of NAR to self-shading, but no corresponding response of PNC is in agreement with PNC for the whole plant of potato, for which PNC, measured 70 days after shade treatment, was not affected by partial shading (Vos & Putten, 2001). In the study by Vos & Putten (2001), LNC of the shaded leaves was only affected at 90% shading but not at 50%, so that very large light reductions are needed before LNC is affected. A minor effect on PNC only at the lowest of the light intensity treatments during the exponential growth phase of crop was also found by Ingestad & McDonald (1989), whereas LNC_a but not LNC of lettuce was affected by shading (De Pinheiro Henrique & Marcelis, 2000). The leaves became thinner (higher SLA) due to shading. So, self shading affects N on an area basis but not N on a basis of mass. Thus, the ontogenetic rate of decline in leaf nitrogen productivity was affected by self shading, whereas PNC was not directly affected.

Leaf N on an area basis (LNC_a)

The average LNC_a of the field-grown cabbage declined with growth (Figure 5 in Paper 1). For cabbage in the climate chamber, the average LNC_a did not decline as expected and LNC_a of the unshaded leaves exposed to constant light was not constant as expected but increased with growth, which was most likely due to luxury consumption. Nevertheless, a vertical gradient in the LNC_a of individual leaves was developed downwards through the canopy parallel to a vertical gradient in photon flux density (Figure 6a and 6b). The light is attenuated with increasing canopy depth because of self shading (Monsi and Saeki, 2005) and LNC_a is adjusted to the incident photon flux density at the leaf surface to maximise photosynthesis (Hirose & Werger, 1987). Maximal LNC_a and photon flux density

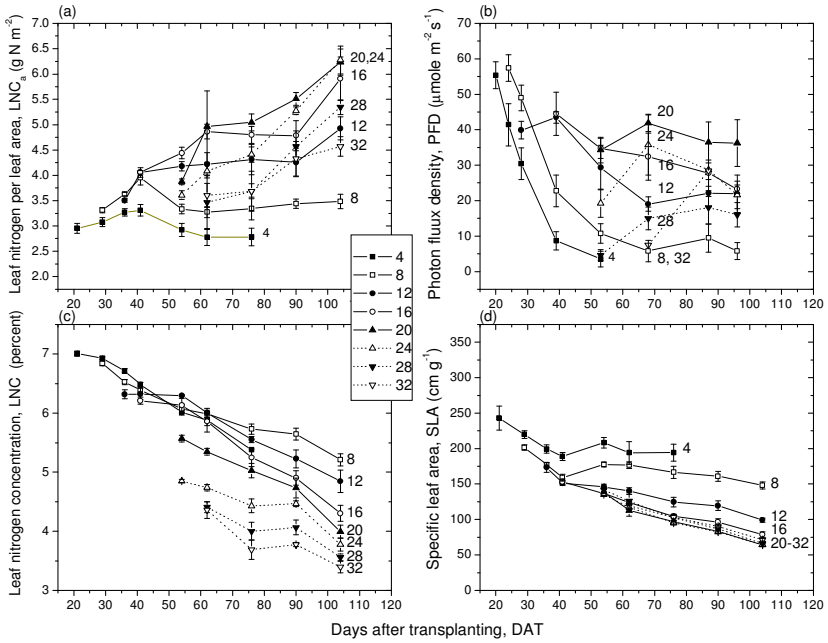

Figure 6. a) Leaf nitrogen concentration on an area basis (LNC_a), b) Photon flux density, (PFD); c) Leaf nitrogen concentration on a weight basis (LNC) and d) specific leaf area (SLA) all for leaves of positions 4, 8, 12, 16, 20, 24, 28 and 32 counted from the bottom of the canopy. Error bars denote ±SE. (=Figure 2 in paper III).

coincided at the same leaf positions (Figure 6a and 6b). The maximal LNC_a and photon flux density were reached for leaves at successively higher leaf positions and finally at leaf position 20 (Figure 6a and 6b). These leaves were characterised by being larger and more horizontally orientated than the more vertical orientated leaves above them. From these leaves LNCa and photon flux density decreased in parallel gradients downwards as well as upwards in the canopy. The decrease in photon flux density at higher leaf positions was a result of their vertical orientation. The decrease in LNCa was a consequence of changes in SLA and LNC (LNCa = LNC/SLA). LNC and SLA were highest at the lowest leaf position (Figure 6c and 6d), LNC was high but the leaves were thin (high SLA) which leads to a low LNCa. Even if LNC decreased upwards in the canopy the increasing thickness (decreasing SLA) caused LNCa to increase. At higher leaf positions, above the leaf positions with maximal LNCa, SLA was fairly constant with leaf position, whereas LNC continued to decrease upwards, which led to decreasing LNCa upwards in the canopy from the leaves with the maximal LNCa. Such an interaction between LNC and SLA leading to a constant LNCa was shown by Charles-Edwards et al., (1987) for forage sorghum. The pattern of LNCa between leaf positions is not clearly shown for the early growth stages in Figure 6a. However, chlorophyll content readings from the U2001 pot experiment at 14, 35 and 49 DAT showed a similar pattern of the relative chlorophyll content between individual leaves with a

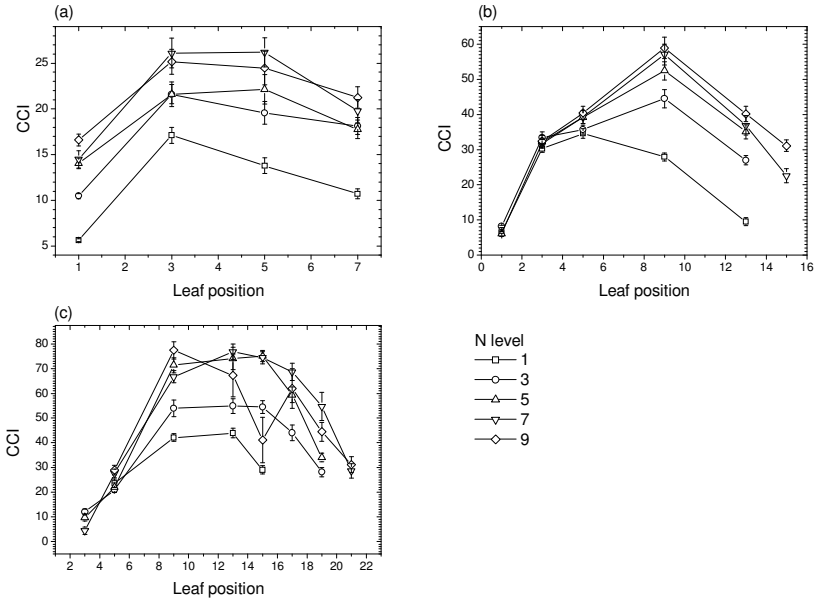

Figure 7. Chlorophyll content index (CCI) for various N application rates as a function of leaf position at a) 14 days after transplantation (DAT) b) 35 DAT and c) 49 DAT. Data from pot experiment U2001. Error bars show ±SE.

maximum in the middle of the canopy (Figure 7). The fully developed horizontally orientated unshaded leaves had maximal LNC_a from young plant stage to a fully developed canopy.

For each sampling date, LNC_a values of those leaves with the highest LNC_a, were plotted against W (Figure 3a in Paper III). The LNC_a of these leaves, that were fully exposed to constant light, increased. A similar trend was shown for chlorophyll (Figure 3b Paper III). The rate of increase in maximal LNC_a was the same as the rate of increase in PNC_a, the scaling coefficient was 0.11 for both of them. The ratio between LNC_a for the leaves fully exposed to light and PNC_a was constant, around 0.8 for whole leaves and slightly lower for punched leaf discs (Figure 4 in Paper III). For young plants, as long as all leaves are unshaded, the LNC_a can be expected to be similar. The ratio of LNC_a of all leaves to PNC_a (leaf nitrogen ratio) of young plants will therefore be similar to the ratio of LNC_a of leaves fully exposed to light to PNC_a during the continued growth. The results in Paper II (Figure 4d) show that the leaf nitrogen ratio of young plants was indeed close to 0.8, similar to the ratio of LNC_a of leaves fully exposed to light to PNC_a. The leaf nitrogen ratio was similar in the field and in the climate chamber (Figure 8), which is in agreement with results of a study on lettuce grown at different light intensity and at varying N supply by De Pinheiro Henriques & Marcelis (2000). There is therefore strong evidence that the critical LNC_a of leaves fully exposed to light for field conditions can be predicted from the critical PNC_a, PNC_{ac}, as $0.8 \times PNC_{ac} = 0.8 \times 4.7 = 3.7$ g N m^{-2}. The hypothesis of a constant LNC_a of unshaded leaves was therefore supported.

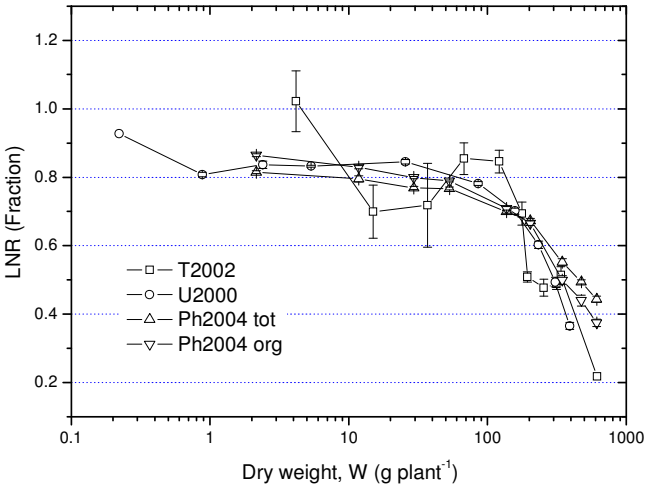

Figure 8. Leaf nitrogen ratio (LNR) of field experiment U2000 and T2002 and of the experiment in the climate chamber Ph2004. Error bars show ±SE.

The results from the field experiment at Ultuna in 2000 provide additional, indirect evidence to support the conclusion that a critical LNC_a can be predicted from PNC_{ac}. Unfortunately LNC of individual leaves was not analysed from data from the field experiments. Therefore, critical LNC of the youngest fully expanded leaves (YFEL) of cabbage cv. Rampo was taken from Table 2 in Huett & Rose (1989). These values of critical LNC were used to estimate critical LNC_a of leaves fully exposed to light under field conditions (Appendix B). It was assumed that the maximal LNC_a is reached at the same leaf positions both under field conditions and in the climate chamber so that values of SLA from the T2002b experiment were selected for the same leaf positions at which LNC_a was maximal in the Ph2004 experiment. The estimated critical LNC_a was 3.5 g N m^{-2} (Table 1) and, except for deviations in weeks 8 and 12, remained constant over 10 weeks. The estimated value of 3.5 g N m^{-2} was based on GR of the lowest N application rate (29 mmol L^{-1}) that gave the highest yield. The estimated LNC_a was slightly lower than the value derived from PNC_{ac} (3.7 g N m^{-2}). The two values of critical LNC_a agree rather well taking into consideration that different cultivars were grown in a quite different climate.

Table 1. *LNC_a for leaves fully exposed to light estimated from LNC of Huett & Rose (1989) and SLA of T2002b at leaf positions, at which LNC_a and PFD had their maximal values in the Ph2004-experiment*

Week	W[1]	SLA[2]	LNC[3]	LNCa[4]
2	8.1	123.5	4.38	3.55
4	22.5	121.8	4.35	3.57
6	57.7	117.5	4.15	3.53
8	124.5	109.3	3.10	2.84
10	206.1	99.2	3.50	3.53
12	265.7	91.9	3.10	3.37

[1] W (g $plant^{-1}$), estimated from GR in Table 1 of Huett & Rose (1989), see appendix B
[2] SLA (cm^2 g^{-1}), estimated from SLA data of T2002b as SLA=124.5 – 0.123 × W, see appendix B
[3] LNC (%), from Table 2 in Huett & Rose (1989)
[4] LNC_a = LNC/SLA (g N m^{-2})

Applications

The results shown in this thesis indicate that assessing the plant N status on the basis of analysis of leaves fully exposed to light as suggested by Lemaire *et al.* (1997) is promising. Matching the actual sampling stage to a critical concentration which precisely corresponds to that growth stage has hitherto been one of the greatest difficulties in plant analysis. This obstacle is removed by leaf area based plant analysis of individual leaves, because the critical concentration remains constant during plant growth and development. Another difficulty has been to choose which part of the plant to sample. For leaf area based analysis, the leaves fully exposed to full light should be sampled. These leaves should be relatively easy to identify and reach, however attention is needed at the sampling to ensure the leaves selected are not shaded at any time during the day. Also the inherent variability between adjacent leaf positions has to be further examined. LNC_a has to be sensitive to variations in N status. A plant adjusts its LA to the N supply in order to maintain LNC_a at a functional level for photosynthesis (Grindlay, 1997). Therefore, it can be expected that LNC_a will not respond to N supply. The response of average LNC_a of cabbage to N supply (Figure 9) and of LNC_a of unshaded maize top canopy leaves to N supply (Lemaire *et al.,* 1997) show however that LNC_a is sensitive to changes in N supply. An appropriate tool has to be developed, such as for example punching tongs for a convenient and rapid sampling of leaf discs with a well-defined leaf area. Rapid sampling is required in commercial practice, and sampling of plants is less time consuming compared to extracting soil cores (Matthäus & Gysi, 2001). Another advantage of plant analysis is that many elements can be diagnosed. Leaf area based plant analysis for diagnostic evaluation of fertiliser strategies has several advantages and seems promising together with the prognostic function of computer-based recommendation systems.

Assessing plant N status on a whole plant basis by relating to W, according to the critical curve derived, has the advantage that each value of PNC_c is uniquely related to a value of W. As with measuring on unshaded leaves, the problem of

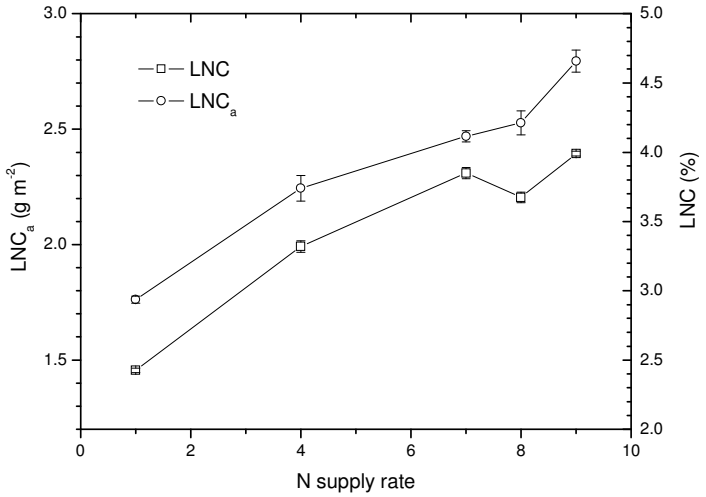

Figure 9. Dependence of leaf nitrogen on an area basis LNC_a (average of canopy) and leaf nitrogen concentration on a weight basis (LNC) on N status. Data at 49 DAT from pot experiment U2001.

defining specific growth stages is avoided. The similar decline in PNC and LAR means that LA measurements from only one level of ample N supply can be used to predict the rate of decline in PNC_c. Only a few repeated samplings are needed to estimate PNC_{ac} from which the parameter a_c for PNC_c can be derived. If W cannot be estimated, for example by sampling in commercial fields, it can be estimated from the number of leaves developed, as was shown in Figure 6 in Paper IV. A universal scale for defining growth stages, the BBCH-code, was developed jointly by BASF, Bayer, Ciba-Geigy and Hoechst (Lancashire *et al.*, 1991). The BBCH-code suggests using the number of leaves developed and diameter of the head to define the growth stage of cabbage (Meier, 1997). Although sampling of whole plants may be of limited use for routine sampling, at least for larger plants, the method is essential in the assessment of plant N status in research and development work. PNC_c is necessary for calculating the optimal N demand of a crop and is basic information needed to develop software for computer-based recommendations. A correct critical concentration is crucial; the error of the calculated accumulated N uptake will increase each day if based on daily calculations from a wrong critical concentration. Increasing PNC with +0.5% adds 100 kg N ha^{-1} more in N-uptake to the 390 kg N ha^{-1} calculated for the PNC_c.

The principles behind a constant PNC_a leading to the same rate (–1/3) of decline of LAR and PNC, which is agreement with the skin-core hypothesis, and the principles behind constant LNC_a of unshaded leaves seem to be general for various crops. However, the –1/3 rate does not generally apply to all crops as is shown by

the varying values of parameter b_{PNC} for different crops (Table 2). The deviation

Table 2. *Values of parameters a_c and b_c of equation 1 for critical PNC for total N of various C_3 and C_4- crops*

Species	*a*	*b*	References
C_3			
White cabbage (*Brassica oleracea* L. var. *capitata* L. *alba* DC.)	5.1	-0.33	Paper 4
Lettuce (*Lactuca sativa* L. Var. *capitata* L.)	4.6	-0.36	Tei *et al.* (2003)
Linseed (*Linum usitatissimum* L.)	4.7	-0.53	Flénet *et al.* (2006)
Pea (*Pisum sativum* L.)	5.1	-0.32	Ney *et al*. (1997)
Potato, (*Solanum tuberosum* L)			
Potato, cv. Russet Burbank	4.6	-0.42	Belanger *et al.,* (2001)
Potato, cv. Shepody	5.0	-0.42	Belanger *et al.,* (2001)
Potato, cv. Bintje & cv. Kaptah Vandel	5.2	-0.56	Duchenne *et al.,* (1997)
Rapeseed (*Brassica napus* L)	4.5	-0.25	Colnenne *et al.* (1998)
Tall fescue (*Festuca arundinacea* Schreb.)	4.8	-0.32	Lemaire & Salette (1984)
Tomato (*Lycopersicon esculentum* Mill.)	4.5	-0.33	Tei *et al.* (2002)
Wheat (*Triticum aestivum* L.)	5.3	-0.44	Justes *et al.,* (1994)
C_4			
Maize (*Zea mays* L.)	3.4	-0.37	Plenét & Lemaire (1999)
Sorghum (*Sorghum bicolour* L.)	3.9	-0.39	Plenét & Cruz (1997)

seems to be caused by special traits of the crop. The linseed crop was originally bred for fibre which explains the steep decline with a b_{PNCc} of –0.53 (Flénet *et al.* 2006). The lower rate of decline in b_{PNCc} (b_{PNCc} of –0.25) of winter oilseed rape was explained by a higher N absorption capacity due to increased root growth in autumn and N reallocation from senescent leaves to the youngest leaves during winter (Colnenne *et al.,* 1998). Further research is needed into the causes of differences between crops and cultivars and to identify possible common traits for crops and cultivars with similar critical concentrations.

The actual N status of cabbage at any time during the growth period can be evaluated by means of the critical concentrations presented. The NNI gives a convenient measure of the N status and is defined as the actual concentration divided by the critical concentration (Lemaire & Gastal, 1997). In the case of analysis of the horizontally orientated unshaded leaves, the same value of critical N concentration can be used during the entire growth period for calculation of the NNI.

The NNI can be used to evaluate the actual plant nutrient management strategy. Such an evaluation is an important component of a nutrient management strategy which tries to as closely as possible match N supply with demand during the entire growing period. Such nutrient management strategies have been made possible through the use of computer-aided decision support tools such as N-Expert and N_Able (Fink & Feller, 1997; Greenwood, 2001). Evaluation will also be called for when more attention is paid to the N supply from manures and residues from the preceding crop (Rahn, 1992; Torstensson & Ekbladh, 2002) as this supply is difficult to predict and therefore best evaluated in terms of its effect on crop N uptake. This is of course of special interest in organic vegetable production. In such production systems crop yields are often lower than in their conventional counterparts and this is often believed to -at least partially- be due to crop nutrient deficiencies. In such systems the farmer often has very little information as to the extent to which the nutrient demand of the crop is met, unless the nutrient status of the crop can be evaluated. Also in research is the nutrient supplying capacity of different organic manures (e.g. legumes, animal manures, etc.) or in different crop rotations often evaluated in terms of their effects on total yield, rather than in terms of their ability to meet crop nutrient demand (e.g. Ögren *et al.,* 1998). At both the research and at the practical level an assessment of the crop nutrient status in terms of nutrient excess or deficiency in relation to potential crop growth would therefore be extremely valuable.

Conclusions

The critical N concentration of cabbage on a whole plant basis was estimated as

$PNC_c = 4.5$ (% of DM) $W < 1.5$ t ha^{-1}

$PNC_c = 5.1W^{-0.33}$ (% of DM) $W > 1.5$ t ha^{-1}

The rate of decline in PNC was characterised by the exponent –0.33. This rate was similar to the rate of decline in LAR for plants grown with ample N supply. The rate of decline in PNC can thus be predicted from that of LAR, but prediction from the regression of LA to W is preferable because pre-linear growth phase data points can also be included in the regression. The rate in LAR changed stepwise following changes from one growth phase to the next (shown for plants in the climate chamber). LAR and PNC declined at similar rates when PNC_a remained constant with growth.

RGR and PNC were constant during the exponential growth phase (with a resulting constant nitrogen productivity according to the nutrient productivity theory). After this phase, RGR and PNC declined at different rates with the difference being equal to the rate of decline in nitrogen productivity and, as PNC_a was constant, equal to the rate of decline in NAR. Thus one growth component of RGR, LAR, declined at the same rate as PNC whereas the other, NAR, made up the difference in the rate of decline between PNC and RGR. The difference in rate of decline in PNC and RGR was most likely mainly due to self shading.

Leaf area based plant analysis on individual leaves appears promising as the critical concentration will be constant during the entire growth period for leaves fully exposed to light. A value of 3.7 g N m^{-2} for white cabbage was derived from the critical PNC_a but the ratio between LNC_a for leaves fully exposed to light and PNC_a has yet to be validated under field conditions.

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Appendices

Appendix A, Relationship of LAR and PNC

According to Caloin & Yu (1984), Greenwood et al. (1991) and Lemaire & Gastal (1997), PNC is linearly related to RGR, equation 2:

$$\mathrm{PNC} = \left(\frac{\mathrm{PNC}_m - \mathrm{PNC}_s}{k} \right) \mathrm{RGR} + \mathrm{PNC}_s$$

The constant k is GR divided by W_m and was assumed to be constant. W_m is the "active" biomass and is mainly located in the leaf. It can be assumed that this type of tissue constitutes a certain proportion of the leaf. When leaf area increases it can therefore be expected that the amount of metabolic dry matter also increases in proportion to the increase of leaf area. NAR decreases due to increasing LA at constant GR during the linear growth phase. If W_m is related to LA rather than to GR, it can be assumed that k decreases at the same rate if metabolic tissues constitute a certain proportion of the leaf. The metabolic processes become less active and less effective although the metabolic tissues remain intact. An analogy between GR to LA and GR to W_m can be assumed as long the proportion of metabolic tissues within the leaf are not reduced by decreasing intercepted radiation:

$$\frac{\mathrm{GR}}{\mathrm{W}_m} = k_2 \times \frac{\mathrm{GR}}{\mathrm{LA}}$$

The equation is rearranged and *W* is inserted on both sides:

$$\frac{\mathrm{W}_m}{\mathrm{W}} = \frac{1}{k_2} * \frac{\mathrm{LA}}{\mathrm{GR}} * \frac{\mathrm{GR}}{\mathrm{W}}$$

Simplified:

$$\frac{\mathrm{W}_m}{\mathrm{W}} = \frac{1}{k_2} \times \mathrm{LAR}$$

Equation 9 in Lemaire & Gastal (1997):

$$\mathrm{PNC} = \frac{\mathrm{PNC}_m \mathrm{W}_m + \mathrm{PNC}_s \mathrm{W}_s}{\mathrm{W}}$$

The pool of structural biomass $W_s = W - W_m$:

$$\mathrm{PNC} = \mathrm{PNC}_m \frac{\mathrm{W}_m}{\mathrm{W}} + \frac{\mathrm{PNC}_s \times \mathrm{W}}{\mathrm{W}} - \mathrm{PNC}_s \times \frac{\mathrm{W}_m}{\mathrm{W}}$$

W_m/W is substituted with $1/k_2 \times \mathrm{LAR}$ and the equation is rearranged:

$$\mathrm{PNC} = \frac{1}{k_2} (\mathrm{PNC}_m - \mathrm{PNC}_s) \times \mathrm{LAR} + \mathrm{PNC}_s \qquad (3)$$

If the assumption of an analogy between GR/W_m and GR/LA is valid, PNC will be linearly related to LAR during the linear growth phase.

Appendix B, Determination of LNC_a from LNC of Huett & Rose (1989)

To estimate LNC_a for leaves fully exposed to light under field conditions data of LNC of the youngest fully expanded leaves (YFEL) from Huett & Rose (1989) were used and data of SLA from the T2002b and Ph2004 experiments. It was assumed that LNC_a was at its maximum at the same leaf positions in the field as in the climate chamber at a given plant size (W). SLA was selected at leaf positions where LNC_a was at its maximum and at a similar W as in the climate chamber. These data of SLA were plotted against W, the linear regression for 20 – 82 DAT was SLA = 124.5-0.123*W, R^2 = 0.983. Data for W that corresponded to the LNC data was not given by Huett & Rose (1989). Therefore, W was estimated from the biweekly data of GR for 29 mmol L^{-1} given in Table 1. Data for the N application rate 29 mmol L^{-1} were used. Decreasing GR during the last weeks suggested use of the logistic growth function:

$$\frac{dW(t)}{dt} = a\,W(t)\left(1-\frac{W(t)}{W_f}\right)$$

The biweekly data of GR were fitted to the derivative with respect to time of the logistic growth function:

$$\frac{dW(t)}{dt} = \frac{a\,W_f\,\exp(-a\,(t-b))}{[1+\exp(-a(t-b))]^2}$$

The parameters were (confidence intervals within parenthesis):
a = 0.54 (0.48 - 0.59), b = 8.77 (8.54 -9.01), W_f = 313 (289-336)
The accumulated growth (W) was calculated from the analytical solution to the logistic growth function with the values of the parameters inserted:

$$W(t) = \frac{313}{1+\exp(-0.54\,(t-8.77))}$$

This expression was used to calculate W corresponding to the LNC of Table 2 in Huett & Rose (1989). The linear relationship (124.5-0.123*W) was used to calculate SLA. LNC_a was finally calculated from the calculated SLA and LNC of Table 2 in Huett & Rose (1989) (LNC_a = LNC/SLA) (Table 2).

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