

# Photosynthesis and leaf-nitrogen dynamics during leaf senescence of tropical maize cultivars in hydroponics in relation to N efficiency in the field

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**Abstract** The selection process of nitrogen (N)-efficient cultivars during plant breeding could be simplified by a specification of secondary plant traits that are decisive for N efficiency. It was shown that leaf senescence under N deprivation of sixteen tropical maize cultivars in a short-term nutrient solution experiment was related to leaf senescence and grain yield under N deficiency (N efficiency) in field experiments. In this study we investigated if a quantification of leaf- and plant-N flows by  $^{15}\text{N}$  labelling can improve the evaluation of genotypic differences in leaf senescence in short-term experiments. Cultivars differed in leaf-N content prior to senescence; however, this appeared to have no significant impact on the development of leaf senescence.

N import into senescing leaves was not related to total plant N uptake, but seems to have been regulated by leaf-inherent factors. Leaf N remaining in the leaf seems to have comprised inefficiently remobilized leaf N, at least during early senescence stages. Photosynthetic rate and chlorophyll contents at early senescence stages depended on additional factors to leaf-N content. Nevertheless, all parameters used to characterize leaf senescence were related to leaf senescence at anthesis in field experiments. However, only photosynthetic rate during late leaf senescence reflected cultivar differences in leaf senescence during reproductive growth and N efficiency in field experiments.

**Keywords**  $^{15}\text{N}$  labelling · Genotypic variation · N deprivation · SPAD · Stay-green · *Zea mays* L.

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## Introduction

N deficiency is among the major abiotic stresses causing yield reductions in maize grown in the tropics (Lafitte and Edmeades 1994b; Bänziger and Lafitte 1997). Since the availability of N fertilizers particularly in sub-Saharan Africa and Latin America is frequently small, the crop productivity has to be improved by a more efficient use of the soil and fertilizer nitrogen supply. The breeding and cultivation of N-efficient cultivars that are defined as forming higher grain yields under N-limiting con-

ditions compared to a standard genotype can play an important role in a sustainable agriculture (Horst et al. 2003; Hirel et al. 2007). Genotypic variability in N efficiency of maize has been shown both in temperate (Moll et al. 1982; Bertin and Gallais 2000; Presterl et al. 2002) and tropical maize (Lafitte and Edmeades 1994a; Akintoye et al. 1999; Kamara et al. 2003). The breeding process of N-efficient cultivars is more efficient, when the selection is performed under low-N target conditions (Bänziger et al. 1997; Presterl et al. 2003). However, with decreasing soil fertility the environmental variability increases and thus heritability for grain yield declines. Therefore, secondary plant traits related to N efficiency have been evaluated for their efficiency as selection traits for N efficiency, since these traits are less prone to environmental variability. In field experiments conducted under low-N conditions it could be shown that delayed leaf senescence (stay-green) is related to high N efficiency (Lafitte and Edmeades 1994a; Bänziger and Lafitte 1997; Worku 2005; Echarte et al. 2008). An alternative approach for the selection of N-efficient cultivars is the screening of seedlings under laboratory conditions for traits related to N efficiency. This approach has several advantages (Teyker et al. 1989): the environmental conditions can be controlled better, a larger number of plants may be tested, and owing to the short experimental period unselected plants can be discarded early. Recently we showed, that leaf senescence of tropical maize seedlings subjected to N deficiency in hydroponics was correlated with leaf senescence and grain yield under N deficiency of the same cultivars in field experiments (Schulte auf'm Erley et al. 2007). Photosynthetic rate during leaf senescence proved to be a better indicator for N efficiency in this study than leaf chlorophyll content. However, photosynthetic rate could explain only up to 20% of the cultivar differences in N efficiency, while leaf senescence in the field experiments could explain 47%.

Since enzymes within the chloroplast stroma are degraded early during leaf senescence, this has been proposed to be responsible for the decline in photosynthetic rate (Hörtensteiner and Feller 2002). Therefore, it is likely that photosynthetic rate during leaf senescence reflects differences between genotypes in leaf-N status, especially under N deficiency-induced leaf senescence. Plant and leaf-N status at the

beginning of the N deficiency period might influence the onset of leaf senescence by alleviating N stress. They are determined by N uptake during early vegetative plant growth and might thus depend upon N supply during that period. An efficient root-N uptake rate during the N depletion period will prolong the N supply to the leaves. Apart from improving leaf-N status, this also increases cytokinin production of the roots (Sattelmacher and Marschner 1978; Wagner and Beck 1993), which will also delay leaf senescence (Buchanan-Wollaston et al. 2003). The leaf-senescence rate might also be influenced by the rate of N export from the leaf. The amount of N exported depends upon the breakdown of N compounds within the leaf and thus protease activity (Brouquisse et al. 2001), but might also be influenced by sink strength (Sinclair and de Wit 1975; Schiltz et al. 2005).

The objective of the present study was to investigate if cultivar differences in N deficiency-induced leaf senescence are dependent on (i) the initial leaf-N content, which may be influenced by the N supply during leaf growth, (ii) the N uptake into the leaf or the total plant after full leaf emergence, or (iii) the amount of N that is exported from the leaf. Clarification of these aspects may help simplifying and/or improving the experimental procedure for an evaluation of N deficiency-induced leaf senescence in short-term experiments as a marker for N efficiency.

## Material and methods

### Plant material and growing conditions

A nutrient solution experiment was conducted in a greenhouse of the Institute for Plant Nutrition at the Leibniz University of Hannover, Germany, from April to June 2005. Sixteen maize cultivars (Table 1) with contrasting N efficiency were cultured at two N levels (N1: 0.1 mM, N2: 0.5 mM) in a completely randomized block design with three replications. Each replication consisted of one pot. The cultivars had been classified by breeders as N-efficient and N-inefficient, which was confirmed by grain yield-determinations under N deficiency in field studies in Kenya and Zimbabwe as described by Worku et al. (2007). Not all of the cultivars that could be

**Table 1** Maize cultivars/hybrids used for the study

No.	Cultivar (Hybrid)	Source	N efficiency	Stay green
I	CML312/CML247//CML78	CIMMYT-Kenya	+	+
II	CML78/CML373	CIMMYT-Mexico	+	+
III	CML395/CML444//CML440	CIMMYT-Zimbabwe	+	–
IV	LPSC4F273-2-2-1-B-B-B/CML202//CML384	CIMMYT-Kenya	+	+
V	CML181/CML182//CML176	CIMMYT-Zimbabwe	+	+
VI	CML395/CML444//CML442	CIMMYT-Zimbabwe	+	–
VII	CML444/CML445//CML440	CIMMYT-Zimbabwe	+	–
VIII	CML444/CML197//CML443	CIMMYT-Zimbabwe	+	+
IX	CML247//CML254	CIMMYT-Mexico	+	+
X	CML442/CML444//[MSRXPL9]C1F2-205-1(OSU23i)-1-1-X-1-X-B-B	CIMMYT-Kenya	+	–
XI	CML202/CML395//CML205	CIMMYT-Zimbabwe	–	–
XII	SC515	Seed-CO-Zimbabwe	–	–
XIII	SC633	Seed-CO-Zimbabwe	–	–
XIV	CML181/CZL01005//CZL01006	CIMMYT-Zimbabwe	–	–
XV	CML264/CML311//CML334	CIMMYT-Mexico	–	–
XVI	CML144/(16304/6303Q)-B-6-1-3-3-B*6	CIMMYT-Mexico	–	–

classified as N-efficient also showed a stay-green trait. Seeds were germinated between moistened filter paper. On 18 April 2005, three seedlings were transplanted into each pot. When the plants developed three visible leaves, they were thinned to two per pot. Pot size was 35.5×31×27 cm with alternately 34 or 47 cm space between pot rows and no space within rows. Nutrient solution containing 500 μM K<sub>2</sub>SO<sub>4</sub>, 100 μM KH<sub>2</sub>PO<sub>4</sub>, 325 μM MgSO<sub>4</sub>, 50 μM NaCl, 8 μM H<sub>3</sub>BO<sub>3</sub>, 1 μM MnSO<sub>4</sub>, 0.4 μM ZnSO<sub>4</sub>, 0.4 μM CuSO<sub>4</sub>, 0.1 μM MoNa<sub>2</sub>O<sub>4</sub>, 85 μM Fe-EDDHA and 1,000 μM CaSO<sub>4</sub> was continuously circulated between 180 L storage containers and twelve 30 L plastic pots connected to each container. Ca(NO<sub>3</sub>)<sub>2</sub> was used as nitrogen source. The N concentration in the nutrient solution was measured daily with nitrate test-strips (Merck, Germany) and both N levels were re-adjusted to the initial level when the concentration of the N<sub>2</sub> treatment (0.5 mM) dropped by half. Plants were grown at 25/15±3°C day/night temperature and a relative humidity of 75% day and night. When leaf 5 (counted from the base of the plant) was fully expanded, one plant per pot was harvested (26 days after germination, DAG). After the first harvest (H1), the nutrient solution was replaced by a solution

containing KNO<sub>3</sub> labelled with 2 atom % <sup>15</sup>N to quantify N uptake before and after full expansion of leaf 5. From 42 DAG, N supply was stopped in order to induce leaf senescence. The second harvest (H2) was initiated when the chlorophyll content of leaf 5 as estimated by SPAD readings (SPAD-502, Minolta, Japan) dropped to zero for the first plants within that N rate (N1: 49 DAG, N2: 51 DAG).

#### Measurements and plant analysis

The chlorophyll contents of leaf 5 and 6 (counted from the base of the plants) were estimated non-destructively with a portable chlorophyll meter (SPAD-502, Minolta, Japan). Three readings per leaf were taken. The measurements were repeated on the same leaf every 2 days between full leaf expansion and the second harvest. Photosynthetic rate of leaf 5 was measured during morning hours immediately before H1 and H2 and photosynthetic rate of leaf 6 was measured before H2 using a portable gas exchange system (LI-6400, LI-COR, Lincoln, NE, USA) with an incoming photosynthetic photon flux density of 1,500 μmol m<sup>-2</sup> s<sup>-1</sup> provided by a red/blue LED light source (6400-02B, LI-COR), and an ambient CO<sub>2</sub> concentration of 400 μmol mol<sup>-1</sup> was

adjusted by the system's CO<sub>2</sub> injector (6400-01, LI-COR). Leaf temperature during the measurements was around 31°C, relative humidity of the air flow amounted to 35 to 40%, which also reflects the conditions for plant growth in the greenhouse. One measurement per leaf was taken on a 2×3 cm leaf segment between mid-length and the top third of the leaf blade avoiding the mid-rib. Apart from CO<sub>2</sub> uptake, transpiration rate and stomatal conductance were simultaneously recorded.

After each harvest, plants were separated into leaf 5, leaf 6, shoot and roots. Leaf area of leaf 5 and 6 was measured using a LI-3100 area meter (LI-COR, Lincoln, NE, USA). All plant samples were dried in an oven at 70°C for >72 h. Dry weights of each component were measured and samples were ground and homogenized for N and nitrate analysis. Nitrogen concentration was determined using a CNS analyzer (Vario EL, Dumas Elementar Analysensysteme GmbH, Hanau, Germany) and the <sup>15</sup>N abundance of the samples was measured with a mass spectrometer (ESD 100, InProcess Instruments, Bremen, Germany). For nitrate analysis 100 mg dry matter was extracted in 10 ml distilled water for 30 min and the extract was analysed using a continuous flow analyzer (San<sup>+</sup>, Skalar, Breda, The Netherlands).

The N content in a specific plant fraction derived from N uptake after full expansion of leaf 5 (new N) was calculated as:

New N(mg)

$$= \left[ \left( {}^{15}\text{N}_{\text{tissue}} - {}^{15}\text{N}_{\text{natural}} \right) / \left( {}^{15}\text{N}_{\text{label}} - {}^{15}\text{N}_{\text{natural}} \right) \right] * \text{N}_{\text{tot}}$$

where <sup>15</sup>N<sub>tissue</sub> is the abundance of <sup>15</sup>N (atom %) in the tissue, <sup>15</sup>N<sub>natural</sub> is the natural abundance of <sup>15</sup>N (0.3663 atom %, Deléens et al. 1994), <sup>15</sup>N<sub>label</sub> is the abundance of <sup>15</sup>N in the nutrient solution (2 atom %) and N<sub>tot</sub> is the total N content in the tissue (mg).

Nitrogen uptake into the shoot between H1 and H2 (shoot new N) was calculated from new N of leaf 5 and 6 plus new N of the remaining shoot. Nitrogen import represents the new N of leaf 5 (g m<sup>-2</sup>), and N export (g m<sup>-2</sup>) represents the difference between leaf-N content at H1 and old leaf N at H2. The latter represents leaf N taken up prior to H1 (old N = total leaf N at H2 minus new leaf N at H2).

Shoot N demand was defined as shoot N at H1 that was necessary for shoot growth between H1 and H2.

It was calculated as shoot growth between H1 and H2 multiplied with shoot N concentration at H2 minus shoot N uptake between H1 and H2.

#### Field data

Data on grain yields and leaf senescence scores under N limiting conditions in the field were taken from Worku (2005). A detailed description of the experiments can be found there and in Worku et al. (2007). Briefly, the experiments were conducted at the CIMMYT research station at Harare, Zimbabwe, (2003 and 2004) and at the Kenya Agricultural Research Institute substation at Kiboko (2003). The cultivars were tested under three N levels (low, medium and high N) at both sites. Since Additive Main effect and Multiplicative Interaction (AMMI) analysis revealed that the Kenya environment was very different from Zimbabwe (Worku et al. 2007), the means of the Zimbabwe experiments were used for the evaluation of the individual cultivars. Data from the nutrient solution experiment were only compared with data under low-N conditions in the field experiments. N deficiency was attained by using non fertilized plots previously depleted of nitrogen. Mean yield reductions at low-N compared to high-N supply were 77 and 70% in Zimbabwe 2003 and 2004, respectively (Worku et al. 2007). Phosphorus and potassium were applied uniformly based on the recommendation for each center prior to planting. The trials at Harare were irrigated to field capacity at planting using sprinkler irrigation. A second irrigation of 20–30 mm was applied 6–7 days after planting to facilitate germination. Thereafter, trials were irrigated to field capacity whenever soil moisture was less than 40% of field capacity. A plot size of 4 m length by 4.5 m width with six rows per plot was used. Spacing was 0.75 m and 0.25 m between rows and plants, respectively. A plant density of 53,333 plants per hectare was kept after thinning. Leaf senescence was scored by visual estimation on a scale of 0 (0% of the plot leaf area senescent) to 10 (100% of the plot leaf area senescent) at anthesis, 14 and 28 days after anthesis. A cultivar was considered as stay-green (Table 1), when its leaf senescence score at low-N was above average 28 days after anthesis in the mean of the Zimbabwe 2003 and 2004 results.

For the determination of grain yield an area of 5.65 m<sup>2</sup> corresponding to 32 plants in the central four rows was harvested immediately after physiological maturity.

### Statistical analysis

Statistical analysis of the data was performed using the PROC GLM procedure of SAS (SAS Institute 2003) testing replication, cultivar, N rate and the cultivar by N rate interaction. The ANOVA was followed by a Tukey test for estimation of the least significant differences between cultivar means. Pearson correlation coefficients were calculated using the PROC CORR procedure of SAS and  $r^2$  values were obtained from Sigma Plot (version 8.0).

## Results

### Plant dry matter and N uptake

Shoot and root dry matter were significantly lower at low N (N1) compared to high N supply (N2) at both harvests (Table 2). Shoot N concentrations at H1 ranged between 47 to 53 mg N (g dry weight)<sup>-1</sup> under high N supply and between 21 to 27 mg N (g dry weight)<sup>-1</sup> under low N (data not shown). After the N depletion period at H2 mean shoot N concentrations were 10 and 15 mg N (g dry weight)<sup>-1</sup> at N1 and N2, respectively. Cultivars differed in shoot and root dry matter within each N rate and these differences were similar for both N rates (no cultivar × N rate interaction). Cultivars VIII and VII had the highest and I and XIII the lowest shoot and root dry matter at H1 in the mean across the N treatments and the ranking of the cultivars remained similar at H2. Shoot N uptake was closely positively correlated with shoot dry matter at H1 (N1:  $r^2=0.92$  at  $P<0.001$ , N2:  $r^2=0.98$  at  $P<0.001$ ) and less closely also at H2 (N1:  $r^2=0.76$  at  $P<0.001$ , N2:  $r^2=0.81$  at  $P<0.001$ ). Cultivar differences in shoot N uptake were not the same between N rates at both harvests (significant cultivar × N rate interaction).

### Leaf parameters

The overall development of leaf senescence during the experiment can be seen from the time course of

SPAD values for six contrasting cultivars (Fig. 1). After the start of the N depletion (42 DAG) SPAD values of leaf 5 decreased under both N rates indicating the beginning of leaf senescence. Slightly delayed, also the SPAD values of leaf 6 decreased. Before the beginning of N depletion there was an increase in SPAD values for some cultivars. In both N treatments the plants were harvested when the first leaves turned yellow. However, mean SPAD values at H2 were higher at N2 compared to N1 (Fig. 1, Table 3).

Before the onset of leaf senescence at H1, all leaf parameters measured showed significantly lower values at N1 than at N2 (Table 3). Cultivars differences were found only in leaf-N contents. These were due to differences in specific leaf weight, while N concentration did not significantly differ between cultivars. Nitrate-N concentrations at H1 comprised between 15% and 30% of the total N concentration at N2, but the cultivar means did not exceed 5% at N1 and at H2 independent on the N treatment.

Between H1 and H2, i.e. during N deficiency-induced leaf senescence, SPAD values, photosynthetic rates, leaf-N contents, N concentrations and especially nitrate-N concentrations decreased for both N rates and leaf positions. Solely the specific leaf weight increased, indicating dry matter accumulation in the leaves during N deprivation.

Significant interactions between cultivar and N rate demonstrating a differential reaction of cultivars to the N rate were found only for SPAD values and nitrate-N concentrations of leaf 6. This was due to the fact that some cultivars (XIII, XI and IV) displayed an earlier drop in SPAD value at N2 than the other cultivars, which was not the case at N1 (data not shown). Leaf nitrate-N concentrations varied between cultivars only for leaf 6 at N2, since in the leaves of the other treatments nitrate-N was nearly completely depleted. Cultivar I had a significantly higher nitrate-N concentration in leaf 6 at N2 than the cultivars IV and XIII.

Relationships between photosynthetic rates, leaf-N contents and SPAD values

Photosynthetic rates and leaf-N contents were positively related (Fig. 2a, b), however, not at each harvest date and for all leaf positions. Most notably

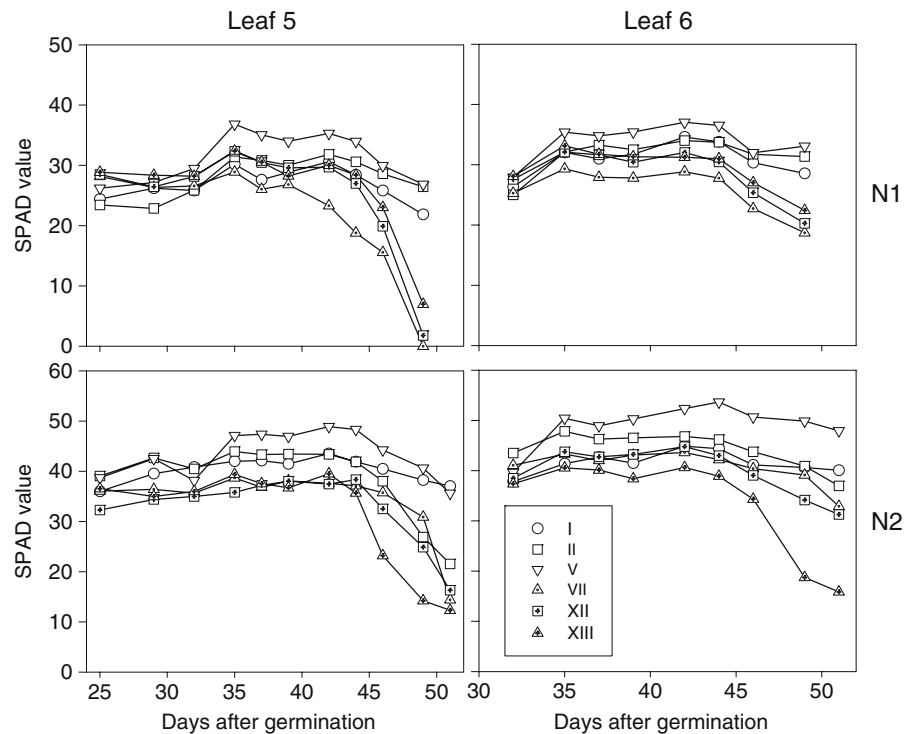
**Table 2** Shoot dry matter, root dry matter, total shoot N uptake and shoot new N uptake (N uptake between the two harvests as calculated from  $^{15}\text{N}$  labelling) of 16 maize cultivars grown in nutrient solution at two N rates (N1, 0.1 mM; N2, 0.5 mM)

with subsequent N depletion from 42 to 51 DAG at harvest 1 (26 DAG) and harvest 2 (N1: 49 DAG, N2: 51 DAG). Cultivars are arranged according to decreasing N efficiency as classified by Worku et al. 2007

N rate	Cultivar	Harvest 1			Harvest 2			
		Shoot dry matter (g)	Root dry matter (g)	Shoot N uptake (mg)	Shoot dry matter (g)	Root dry matter (g)	Shoot N uptake (mg)	Shoot new N uptake (mg)
N1	I	1.15	0.54	29.1	12.9	4.3	172	115
	II	1.88	0.98	44.6	18.6	6.6	267	182
	III	1.87	1.12	48.1	23.9	8.2	280	188
	IV	1.45	0.62	30.7	16.7	5.3	219	138
	V	2.19	0.95	55.5	20.8	5.9	238	157
	VI	2.42	1.19	52.6	18.0	6.8	180	103
	VII	2.35	1.35	57.9	18.1	7.5	197	119
	VIII	2.71	1.31	61.1	27.2	8.6	353	245
	IX	1.19	0.70	31.5	14.7	4.9	187	129
	X	1.81	0.83	46.6	17.4	6.2	222	153
	XI	1.78	0.71	41.4	22.5	6.4	301	182
	XII	1.52	0.56	35.5	14.9	5.4	204	136
	XIII	1.48	0.53	37.2	16.7	5.8	236	145
	XIV	1.68	0.81	43.9	22.8	6.6	356	265
	XV	1.32	0.71	34.3	16.8	5.4	186	118
	XVI	1.68	0.70	41.0	17.5	6.0	230	159
		Mean	1.78	0.85	43.2	18.7	6.2	239
	LSD <sub>0.05</sub>	1.27	0.76	31.8	12.8	4.1	219	181
N2	I	1.30	0.34	67.4	31.5	7.6	674	515
	II	2.72	0.69	138.4	46.4	12.5	818	589
	III	2.82	0.82	137.2	52.1	14.2	952	699
	IV	1.71	0.39	83.9	33.5	9.2	516	342
	V	2.60	0.60	135.7	44.4	9.7	802	604
	VI	2.76	0.74	134.6	55.1	11.8	1,118	844
	VII	3.18	0.66	165.7	57.0	15.2	1,194	906
	VIII	2.88	0.65	136.6	56.1	13.7	1,115	844
	IX	1.80	0.42	93.1	36.9	8.4	721	547
	X	1.93	0.44	95.0	46.8	11.5	923	699
	XI	3.07	0.57	148.0	37.2	11.6	523	343
	XII	1.77	0.35	91.3	48.2	10.2	1,035	789
	XIII	1.24	0.28	63.1	35.0	9.8	544	368
	XIV	2.49	0.56	118.3	47.5	11.9	903	681
	XV	2.67	0.69	127.1	50.6	9.6	771	572
	XVI	2.07	0.58	98.1	41.4	10.8	846	662
		Mean	2.31	0.55	114.6	45.0	11.1	848
	LSD <sub>0.05</sub>	1.25	0.32	65.0	27.4	8.9	765	661
	Cultivar	**	**	**	**	**	*	*
	N rate	**	**	**	**	**	**	**
	Cult. × N	ns	ns	**	ns	ns	*	*

LSD, least significant difference between cultivars at  $P < 0.05$ ns denotes non significant, \* $P < 0.05$ , \*\* $P < 0.001$

**Fig. 1** SPAD values of leaf 5 (left column) and leaf 6 (right column) of 6 maize cultivars grown in nutrient solution at two N rates (upper row: N1, 0.1 mM; lower row: N2, 0.5 mM) with subsequent N depletion from 42 to 51 DAG



the relationship did not hold true across harvest dates and leaf positions, especially at N1. The decrease in photosynthetic rate was stronger than in leaf-N contents, which even increased with ongoing leaf development for some cultivars at N1 (Fig. 2a). Photosynthetic rates and leaf-N contents were more closely related at late stages of leaf senescence, while no correlation could be found before the onset of leaf senescence.

Also photosynthetic rates and SPAD values did not decrease in parallel during leaf senescence; the decrease in photosynthetic rate was stronger than in SPAD (Fig. 2c, d). The correlations between photosynthetic rate and SPAD were less close than between photosynthetic rate and leaf-N content at a very late stage (leaf 5, N1) but not at an earlier stage (leaf 6, N2) of leaf senescence.

SPAD values and leaf-N contents were generally closely correlated (Fig. 2e, f), at N2 even before the onset of leaf senescence (Harvest 1). However, the relationship varied between harvest dates and leaf positions. SPAD values of leaf 6 were comparatively higher in relation to leaf-N content than SPAD values of leaf 5.

#### Leaf-N dynamics

Although there were only small net changes in leaf-N content between H1 and H2 at N1, considerable N amounts were exported from and imported into the leaf during this time span (Table 4). Leaf-N contents before the onset of leaf senescence at H1 were more than two times higher at N2 compared to N1. The amount of N exported between H1 and H2 was nearly four times higher at N2 compared to N1. Nevertheless, the remaining N in the leaf at H2 (leaf N old) and total leaf N at H2 were significantly higher at N2 than at N1. These results correspond with the higher SPAD values and photosynthetic rates at H2 under N2 and reflect a generally lower degree of senescence at the time of harvest for the plants grown at N2. N import into leaf 5 did not differ between N rates, although total shoot N uptake between H1 and H2 was higher at N2 than at N1.

Cultivars differed in leaf-N contents already at H1, with cultivar IV having a lower leaf-N content than some of the other cultivars. Although cultivar differences in leaf-N contents were more pronounced at N2, cultivar IV also tended to have the lowest leaf-N

**Table 3** Mean SPAD value, photosynthetic rate (PR;  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), leaf N ( $\text{g m}^{-2}$ ), specific leaf weight (SLW;  $\text{g m}^{-2}$ ), N concentration ( $\text{mg g}^{-1}$ ) and nitrate-N concentration ( $\text{mg g}^{-1}$ ) of leaf 5 and 6 of 16 maize cultivars grown in nutrient solution at two N rates (N1: 0.1 mM, N2: 0.5 mM) with subsequent N depletion from 42 to 51 DAG at harvest 1 (26 DAG) and harvest 2 (N1: 49 DAG, N2: 51 DAG)

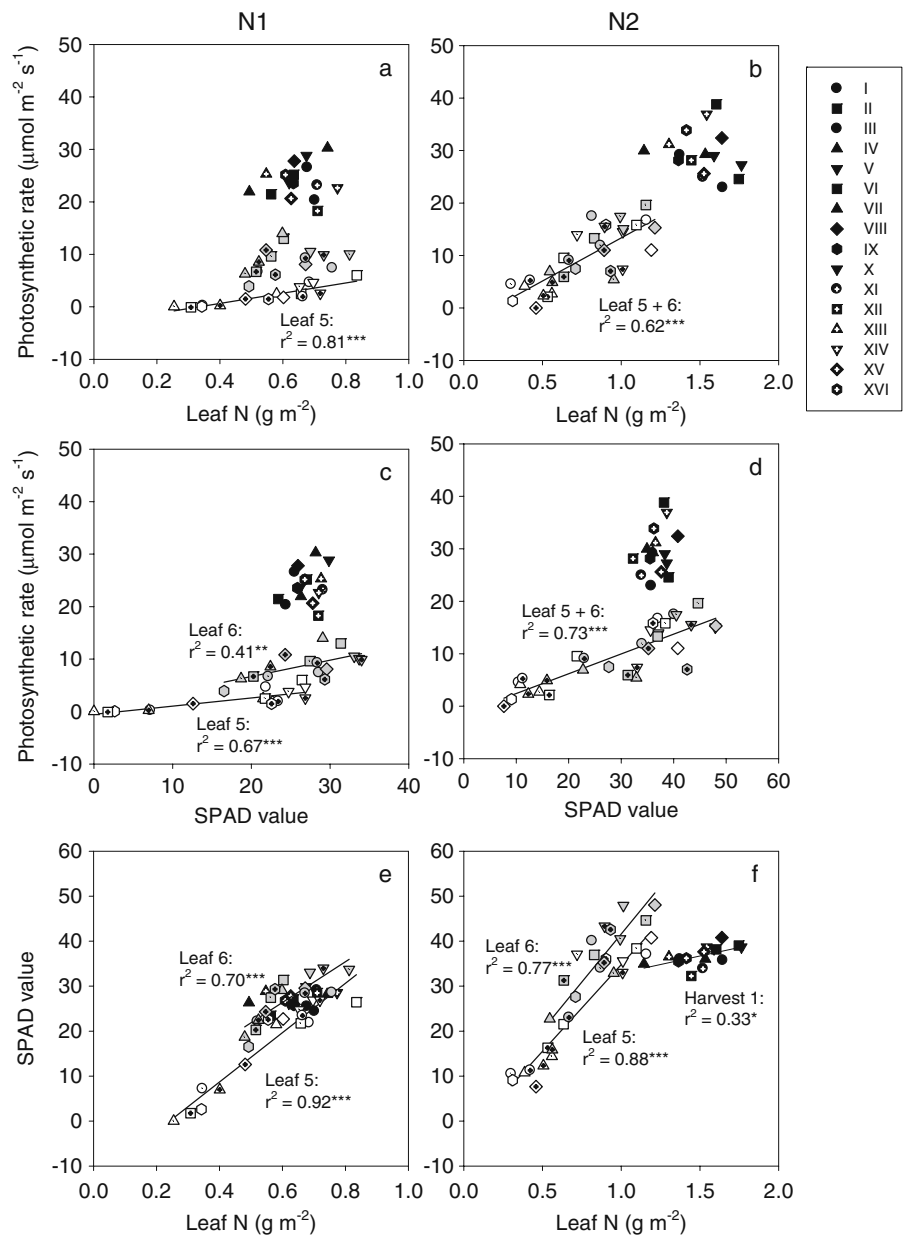
		Harvest 1		Harvest 2	
		Leaf 5		Leaf 5	Leaf 6
SPAD	N1	27.0		16.9	26.8
	N2	36.8		23.2	35.4
	Cultivar	ns		***	***
	N rate	***		**	***
	Cult. $\times$ N	ns		ns	*
PR	N1	24.0		2.1	8.8
	N2	29.5		7.9	11.5
	Cultivar	ns		***	**
	N rate	***		***	**
	Cult. $\times$ N	ns		ns	ns
Leaf N	N1	0.65		0.55	0.61
	N2	1.51		0.70	0.86
	Cultivar	**		***	*
	N rate	***		**	***
	Cult. $\times$ N	ns		ns	ns
SLW	N1	27.0		44.9	38.1
	N2	30.0		35.9	35.9
	Cultivar	***		ns	ns
	N rate	***		***	ns
	Cult. $\times$ N	*		ns	ns
N conc.	N1	24.0		12.3	16.2
	N2	50.4		19.4	24.5
	Cultivar	ns		***	***
	N rate	***		***	***
	Cult. $\times$ N	ns		ns	ns
Nitrate-N	N1	0.34		0.03	0.03
	N2	11.4		0.38	0.56
	Cultivar	ns		ns	*
	N rate	***		***	***
	Cult. $\times$ N	ns		ns	*

ns denotes non significant

\* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$



**Fig. 2** Relationships between photosynthetic rates and leaf-N contents (**a, b**), between photosynthetic rates and SPAD values (**c, d**), and between SPAD values and leaf-N contents (**e, f**) of 16 maize cultivars grown in nutrient solution at two N rates (left column: N1, 0.1 mM; right column: N2, 0.5 mM) with subsequent N depletion from 42 to 51 DAG. *Black symbols*: leaf 5 at harvest 1; *white symbols*: leaf 5 at harvest 2; *gray symbols*: leaf 6 at harvest 2. \*, \*\*, \*\*\* = significant at  $P < 0.05$ , 0.01 and 0.001, respectively



content at N1. These cultivar differences in leaf N at H1 were not conserved during senescence. Cultivar differences in leaf N old and leaf N at H2 did not correspond to the cultivar differences found in leaf N at H1. Instead, leaf N at H1 influenced the amount of N export. In principle, cultivar differences in N export were mainly related to those in leaf N old; however, for cultivars having the same amount of leaf N old, N export was higher for cultivars that had a high leaf N at H1 under N1. Cultivar differences in leaf N old

were more pronounced under N1 than N2. Cultivar II had a significantly higher leaf N old than cvs VII, IX and XII at N1. No cultivar differences in leaf N old could be found under N2 owing to a high variability of this parameter. However, there was a significant shift in cultivar ranking between N1 and N2, indicating a differential cultivar reaction in this parameter in dependence on the rate of N supply. A significant cultivar by N rate interaction was also found in N export, but not in leaf N at H1. Cultivars

**Table 4** Leaf N ( $\text{g m}^{-2}$ ) at harvest 1 (H1), N export ( $\text{g m}^{-2}$ ), Leaf N of H1 still present in the leaf at harvest 2 (Leaf N old;  $\text{g m}^{-2}$ ), N import ( $\text{g m}^{-2}$ ) and leaf N ( $\text{g m}^{-2}$ ) at harvest 2 (H2) of 16 maize cultivars grown in nutrient solution at two N rates (N1, 0.1 mM; N2, 0.5 mM) with subsequent N depletion from 42 to 51 DAG at harvest 1 (26 DAG) and harvest 2 (N1: 49 DAG, N2: 51 DAG)

N rate	Cultivar	Leaf N H1	N export	Leaf N old	N import	Leaf N H2
N1	I	0.70	0.23	0.48	0.21	0.68
	II	0.56	0.00 b	0.57 a	0.27 a	0.83 a
	III	0.68	0.41	0.27	0.08	0.35 b
	IV	0.49	0.09	0.41	0.17	0.58
	V	0.62	0.13	0.49	0.21	0.70
	VI	0.63	0.12	0.51	0.15	0.66
	VII	0.74	0.53 a	0.21 b	0.04 b	0.25 b
	VIII	0.64	0.23	0.41	0.20	0.60
	IX	0.63	0.38	0.25 b	0.09	0.34 b
	X	0.68	0.26	0.41	0.24 a	0.65
	XI	0.71	0.24	0.47	0.19	0.66
	XII	0.71	0.48 a	0.24 b	0.07	0.31 b
	XIII	0.55	0.23	0.32	0.08	0.40
	XIV	0.77	0.27	0.51	0.21	0.72
	XV	0.63	0.27	0.35	0.13	0.48
	XVI	0.61	0.26	0.35	0.20	0.55
		Mean	0.65	0.26	0.39	0.16
	LSD <sub>0.05</sub>	0.43	0.45	0.31	0.20	0.48
N2	I	1.37	0.49 b	0.88	0.28	1.16
	II	1.75 a	1.27	0.48	0.16	0.63
	III	1.65	1.41 a	0.24	0.06	0.30
	IV	1.14 b	0.84	0.30	0.09	0.39
	V	1.76 a	1.04	0.72	0.29	1.01
	VI	1.60	0.79	0.81	0.29	1.10
	VII	1.53	1.10	0.43	0.13	0.56
	VIII	1.64	0.76	0.88	0.31	1.19
	IX	1.36	1.12	0.25	0.06	0.31
	X	1.59	1.18	0.53	0.19	0.72
	XI	1.52	1.19	0.33	0.09	0.42
	XII	1.45	1.03	0.41	0.12	0.53
	XIII	1.30	0.90	0.39	0.12	0.51
	XIV	1.54	0.78	0.76	0.24	1.01
	XV	1.53	1.19	0.34	0.12	0.46
	XVI	1.41	0.74	0.67	0.23	0.90
		Mean	1.51	0.99	0.53	0.17
	LSD <sub>0.05</sub>	0.51	0.82	0.66	0.31	0.95
	Cultivar	**	***	***	***	***
	N rate	***	***	***	ns	**
	Cult. × N	ns	*	*	ns	ns

LSD, least significant difference between cultivars at  $P < 0.05$ . Values denoted by different letters are significantly different at  $P < 0.05$  within each N rate

ns denotes non significant, \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$

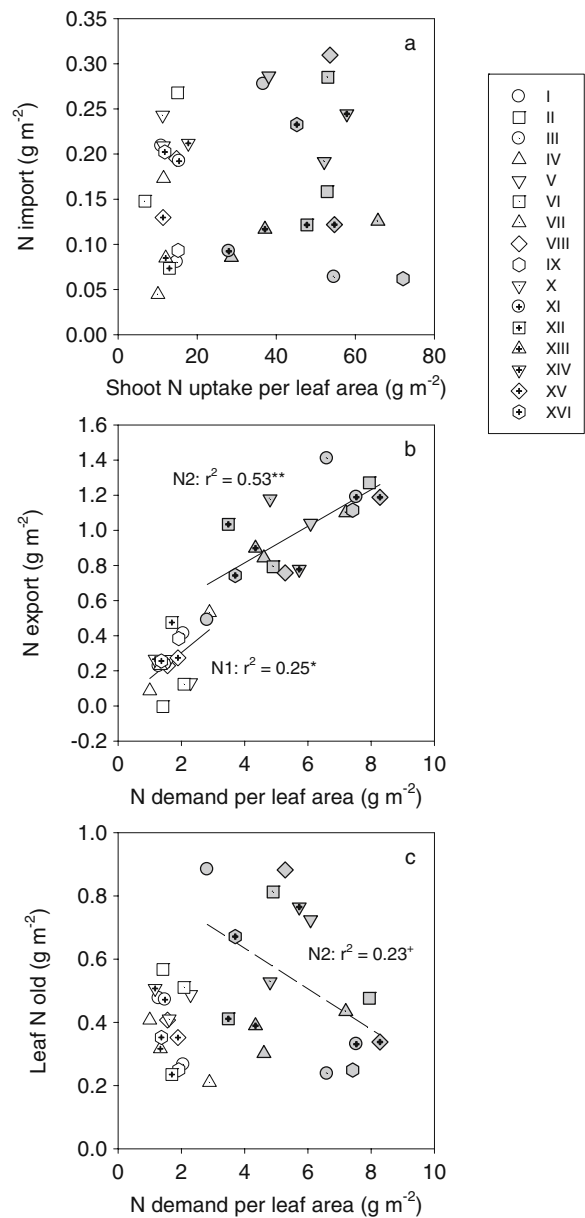
also differed in N import at N1. The cultivar differences were similar, but not entirely the same as for leaf N old. Cultivar X had a high N import, although leaf N old was not high for this cultivar. At N2 no cultivar differences were detected in N import, which was clearly due to the very high variation in this trait at N2. Therefore, also no interaction between cultivar and N rate was found. Cultivar differences in total leaf N at H2 were the same as for leaf N old, since this was also the main quantitative fraction of leaf N at H2, compared to a lower amount of N import.

#### Relationship between leaf-N dynamics and plant-N dynamics

It might be expected that N import and N export from a senescing leaf is not only influenced by cultivar differences in leaf senescence but also by shoot N uptake and N demand for ongoing shoot growth under N deprivation. In evaluating these relationships it has to be considered that cultivars with a higher shoot biomass and thus N uptake were also characterized by a higher area of leaf 5 which has to be supplied with N and from which N can be withdrawn. Therefore, shoot N uptake and N demand for retranslocation are presented per unit area of leaf 5 in Fig. 3. As could already be seen from Table 4, N import was not higher at N2 compared to N1, although total shoot N uptake between H1 and H2 was higher per unit leaf area of leaf 5 (Fig. 3a). Cultivar differences in N import were also not related to shoot N uptake, since differences between cultivars in shoot N uptake per unit leaf area within both N rates were not significant ( $P>0.1$ ). In contrast, N export was related to the N amount that had to be retranslocated to support new shoot growth, especially at N2 (Fig. 3b). According to the higher N export shoot N demand for retranslocation was higher at N2 compared to N1 ( $P<0.001$ ). The leaf N remaining in the leaf at H2 (leaf N old) was not related to the shoot N demand at N1 and only slightly negatively related at N2 (Fig. 3c).

#### Relationship between leaf parameters in nutrient solution and leaf senescence scores and grain yield in field experiments

SPAD values, leaf N and photosynthetic rate at H2 which were used as senescence parameters in the



**Fig. 3** Relationships between N import and shoot N uptake per unit leaf area of leaf 5 (a), between N export and shoot N demand per unit leaf area of leaf 5 (b), and between leaf N old and shoot N demand (c) of 16 maize cultivars grown in nutrient solution at two N rates (white symbols: N1, 0.1 mM; gray symbols: N2, 0.5 mM) with subsequent N depletion from 42 to 51 DAG. +, \* and \*\* = significant at  $P<0.1$ , 0.05 and 0.01, respectively

nutrient-solution experiment, reflected the cultivar differences in the degree of leaf senescence at anthesis in the field experiments in Zimbabwe (Table 5). However, this was true only for leaf 5 in the nutrient

**Table 5** Simple linear correlation coefficients for the relations between SPAD, leaf N ( $\text{g m}^{-2}$ ) and photosynthetic rate (PR;  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) of leaf 5 and 6 at harvest 2 of plants grown in nutrient solution and leaf senescence score at anthesis and 28 days after anthesis, and grain yield in the field of 16 maize

cultivars. The plants were grown in nutrient solution at two N rates (N1, 0.1 mM; N2, 0.5 mM) with subsequent N depletion from 42 to 51 and in the field under severe N stress in Zimbabwe at 2003 and 2004

	N1		N2	
	Leaf 5	Leaf 6	Leaf 5	Leaf 6
Leaf senescence score at anthesis				
SPAD	-0.43 <sup>+</sup>	-0.38	-0.49 <sup>+</sup>	-0.43 <sup>+</sup>
Leaf N	-0.45 <sup>+</sup>	-0.40	-0.46 <sup>+</sup>	-0.20
PR	-0.62*	-0.19	-0.56*	-0.42
Leaf senescence score 28 days after anthesis				
SPAD	-0.28	-0.18	-0.18	-0.26
Leaf N	-0.40	-0.24	-0.28	-0.01
PR	-0.61*	-0.18	-0.30	-0.29
Grain yield				
SPAD	0.09	0.05	0.02	0.04
Leaf N	0.22	0.17	0.04	0.01
PR	0.46 <sup>+</sup>	0.24	0.16	-0.08

<sup>+</sup>  $P < 0.1$  and \* $P < 0.05$  ( $n = 16$ )

solution experiment, while parameters from leaf 6 were hardly significantly correlated with leaf senescence score from the field experiments. Photosynthetic rates of leaf 5 were closer related to leaf senescence scores in the field experiments than SPAD values or leaf N. Most of the senescence parameters in the nutrient solution experiment were not suited to characterize leaf senescence score during reproductive growth in the field experiments. An exception was photosynthetic rate of leaf 5 at N1 which was significantly negatively related to leaf senescence score 28 days after anthesis in the field. Photosynthetic rate of leaf 5 at N1 was also weakly correlated with grain yield under low-N in the field reflecting the N efficiency of the cultivars.

## Discussion

This study was performed to quantify the importance of leaf and plant-N fluxes for the development of leaf senescence.

It may be assumed that leaf-N contents prior to leaf senescence might have an impact on senescence. Although cultivars differed in leaf-N content after full leaf expansion, i.e. at H1 (Tables 3, 4), these differences appeared to be not important for cultivar differences in leaf-N content during leaf senescence at H2 (Table 4). Instead, higher leaf-N contents at H1 increased N export. Therefore, it seems that the initial leaf-N contents at H1 influenced the N availability in the leaves for N export but not the breakdown of N compounds in the leaf. Obviously, under sufficient N supply, N pools are formed in the leaf, which can easily be retranslocated even without physiological degradation of leaf compounds. Thus, different from the initial expectation, cultivar differences in leaf-N contents at H1 did not lead to a corresponding delay in leaf senescence.

Unexpectedly, N import represented a decisive part of total leaf-N even after full leaf expansion, and cultivar differences in N import were also important for differences in total leaf-N at H2 (Table 4). Since N import was not related to total plant N uptake

(Fig. 3a), it was probably governed by leaf-inherent factors. Nitrate-N enters the leaf by the transpiration stream, thus, a decrease in stomatal conductance might have determined N import. Stomatal aperture during leaf senescence may be influenced by changes in photosynthetic rate (Wong et al. 1985) or by abscisic acid (Chapin et al. 1988; He et al. 2005). On the basis of the presented data it cannot be decided whether stomatal conductance might play a regulatory role in the development of leaf senescence or if it merely reflects differences in leaf senescence or photosynthetic rate. In any case, a clear relationship between plant N uptake and leaf senescence that is regulated via N import into the leaf could not be established. Probably, N uptake regulates leaf senescence primarily through signalling factors, like cytokinins (Gan and Amasino 1997), ABA (Weaver et al. 1998) or nitrate influx (Crafts-Brandner et al. 1984) into the leaf. In this context, the timing of N deprivation might be more important than the N uptake rate while N is still available.

It was further assumed that N export reflects either breakdown processes of N compounds in the leaf or sink strength for N assimilates. Since N export seems to have been influenced by N availability in the leaves prior to leaf senescence (at H1; Table 4), leaf N old may reflect better the breakdown of N compounds in the leaf and its subsequent retranslocation. N export from the leaf correlated with N demand for shoot growth (Fig. 3b). Thus, N demand may have determined N export; however, it may be more likely that a high N export due to a high N availability supported shoot growth and a high shoot N concentration. Leaf N old, in contrast, was slightly correlated with shoot N demand only at N2 (Fig. 3c). This might have been caused by an inefficient N retranslocation from leaf 5 at N2 rather than by influences of sink N-demand on N-breakdown processes in the leaf, since such effects should become apparent also under N1. It seems unlikely that N is remobilized inefficiently under conditions of strong N depletion. However, it has to be considered that also shoot growth and thus sink size is inhibited under these conditions. Leaf area growth is strongly decreased by N deficiency (Sinclair and Horie 1989; Lawlor et al. 2001; Vos et al. 2005). A poor leaf growth will lead to a low carbohydrate demand and a low phloem-sap flow and thus N flux from matured leaves to the sink. Such effects could modify the relationship between leaf senescence and

leaf-N content. However, at least leaf N old at N1 seems to have reflected the breakdown of N compounds in the leaf quite well. In this case, initial leaf-N contents at H1 were lower and leaf senescence was already more progressed. The fact that cultivar ranking in leaf N old shifted between N1 and N2 (Table 4) suggests that cultivars differed specifically in degradation steps during late senescence that were either not visible or not present during earlier senescence stages. These differences might be caused by differences in the activity of specific proteases. E.g., a mutation in the *See2 $\beta$*  gene of maize coding for a cysteine protease was found to lead to differences between mutants in late leaf senescence (Donnison et al. 2007). This mutation even led to a higher grain yield of the mutants under limiting N supply, which demonstrates the importance of this protease.

Another underlying assumption of this study was that under N deprivation leaf-N fluxes have a major influence on the induction and development of leaf senescence and thus also on photosynthetic rate and leaf chlorophyll contents during this time period. However, the obtained results show that the decrease of photosynthetic rate after full leaf expansion and the development of leaf chlorophyll content were influenced by additional traits.

The decrease in photosynthetic rate after subjecting the plants to N deficiency was not necessarily related to a concomitant decrease in leaf-N content (Fig. 2). A close correlation between photosynthetic rate and leaf-N content was only found at late stages of leaf senescence (Fig. 2a). Similar results have been found for maize hybrids during the grain filling period in field experiments (McCullough et al. 1994; Dwyer et al. 1995). Also here, decreases in photosynthetic rates were found that could not be attributed to decreases in leaf-N content. In many cases the degradation of N-containing enzymes within the chloroplast stroma during early leaf senescence has been proposed to be responsible for the decline in photosynthetic rate (Buchanan-Wollaston 1997; Hörtensteiner and Feller 2002). In field experiments investigating maize hybrids differing in N efficiency, the efficiency of the electron transport in the light reactions seemed to have been responsible for the decline in photosynthetic rate following N deprivation (Echarte et al. 2008). On the other hand a declining photosynthetic rate has also been suggested to induce leaf senescence and consequently also N remobilisation from the leaf

(Hensel et al. 1993). In this study, the decrease in photosynthetic rate might have been caused by a negative feedback regulation due to an accumulation of C assimilates in the leaves. The increase in specific leaf weight (Table 3) shows that a strong dry matter accumulation occurred in the leaves during N deficiency. Apart from this, it might also be possible that N compounds were broken down in the leaves but inefficiently remobilised. However, since SPAD values did not decline very much stronger than leaf-N contents (Fig. 2e, f), a strong breakdown of chloroplasts seems to be unlikely.

Also the relationship between SPAD values and leaf-N content was not constant between leaf positions and harvest times (Fig. 2e, f). SPAD values still increased for some cultivars after full leaf expansion as long as N was available (Fig. 1). The reason was probably that a higher investment into chlorophyll enables the plants to adapt to the shading of older leaves during ongoing plant development (Evans 1989). Consequently, SPAD did not equally reflect leaf-N content in all cases.

Therefore, it has to be concluded that all parameters used to characterize leaf senescence in this study—leaf-N content, photosynthetic rate and SPAD values—did not fully reflect the status of leaf senescence, i.e. the physiological degradation of the leaf, but also factors apart from this.

The correlations between the leaf-senescence parameters measured in hydroponics and the field results show that all senescence parameters reflected cultivar differences in leaf senescence in the field until anthesis (Table 5). This occurred despite the fact that the development of SPAD values, leaf N and photosynthetic rate during leaf senescence were influenced by different factors. Therefore, all parameters reflected—at least partly—genotypic differences in leaf senescence that were independent of the specific growing conditions. This is in contrast to other results, where genotypic differences in stay-green of maize could be measured only under continued N supply (Subedi and Ma 2005).

The parameters measured at later stages of leaf senescence (leaf 5) reflected leaf senescence in the field experiments better than the parameters measured at early stages (leaf 6). This means that either cultivar differences in senescence induction were distinctly different from those in the field experiments, or the parameters used to measure leaf senescence in

hydroponics were influenced too much by the specific growing conditions at early senescence stages. Especially during early senescence, when cultivar differences are still small, such effects will have an impact on the results.

Hardly any of the parameters measured in hydroponics correlated with leaf senescence score in the field at 28 days after anthesis. This means that cultivar differences in leaf senescence during reproductive growth can hardly be reproduced in a short-term nutrient solution experiment. Several differences between vegetative and reproductive growth might influence the induction and development of leaf senescence: Also if leaf senescence is induced by N shortage both in hydroponics and under field conditions, the timing of N shortage is dependent upon different factors. In the field, the exploration of N sources in deeper soil layers might play the most important role for N uptake during reproductive growth (Wiesler and Horst 1994). Thus in the field, root growth and morphology are the most important plant traits, which play only a minor role for N uptake in hydroponics. Second, source-sink relationships differ distinctly between vegetative and reproductive growth, both for carbohydrates and as a consequence also for nitrogen. The changes in assimilate flows might influence the development of leaf senescence, or at least the parameters used to characterize leaf senescence. In addition, light conditions differ between the greenhouse and open-air conditions and between single plants and plants in a canopy. This might have consequences for leaf chlorophyll contents and possibly also for photosynthesis and total leaf-N. Nevertheless, photosynthetic rate during late stages of leaf senescence (leaf 5 at N1) was significantly correlated to leaf senescence in the field experiments and to a lower degree also to N efficiency (Table 5). This suggests that cultivar differences in specific steps of leaf senescence related to the breakdown of the photosynthetic apparatus contribute to N efficiency in the field.

In conclusion, the results of the study show that the characterization of N deficiency-induced leaf senescence in short-term nutrient solution experiments might be useful for the selection of N-efficient maize cultivars. However, this experiment also highlighted difficulties in comparing leaf senescence of seedlings grown in hydroponics to plants during reproductive growth in field experiments. Since leaf senescence is a complex process prone to many influencing factors,

it will be necessary to investigate the different underlying mechanisms in more detail in order to be able to better specify the genetic base of senescence. The results of this study suggest that degradation steps of the photosynthetic apparatus during late leaf senescence might be promising candidates.

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