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Utility of proximal plant sensors to support nitrogen fertilization in *Chrysanthemum*

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ABSTRACT

Chrysanthemum morifolium Ramat. is a commonly grown ornamental worldwide. A proper timing of nitrogen (N) supply is essential for a qualitative crop and the return on investment for growers. Sub-optimal nitrogen nutrition negatively influences the commercial plant quality, while supra-optimal N has an environmental impact due to nitrate leaching. Therefore, (a) reliable indicator(s) of plant nitrogen status is/are needed. Two field-grown potted *Chrysanthemum* cultivars, 'Maya' and 'Orlando' were studied for three consecutive years (2016–2018). Three different N treatments were applied in order to obtain a variation in N content. Plant quality measurements consisted of plant height, diameter, leaf mass per area (2017 and 2018 only), biomass and foliar and plant N content analysis. Optical measurements were performed with a SPAD sensor (2016 and 2017) and a Dualex Scientific sensor (2017 and 2018) on leaf level and with a GreenSeeker NDVI meter on canopy level. Biomass, height and diameter tended to be smaller in the minimal fertilizer treatments. Leaf mass per area did influence the relation between N and chlorophyll measured with SPAD and Dualex. Epidermal polyphenolics measured with Dualex correlated better with foliar nitrogen than non-destructive chlorophyll measurements and the nitrogen balance index. Since abaxial epidermal polyphenolics were highly correlated with foliar nitrogen and convenient to measure in-field, we propose this measurement for decision support in *Chrysanthemum* fertilization. Because of cultivar and sometimes year-to-year variability, reference plots can be of help for growers and advisors. NDVI was found to be more susceptible for yearly variation, but very high correlation with several quality parameters and convenience in use make this vegetation index useful for detecting the extent of spatial quality variability and thus support site dependent N requirements to reach the desired plant diameter at the end of the growing season.

1. Introduction

Chrysanthemum morifolium Ramat. is one of the most commonly grown ornamental species worldwide (Mol et al., 1995; Royal FloraHolland, 2016; Xia et al., 2006). The phenotype of high quality pot chrysanthemum is a hemispherical shape completely covered with flowers (cushion type) and rich green leaves. Timing nitrogen (N) application during the outdoor production phase, to supply adequate N when the crop needs it, will reduce the environmental impact while a high plant quality will be maintained.

Nitrogen is the second most essential element within plants, only bypassed by carbon. N functions as a constituent of proteins, nucleic acids, chlorophyll and other cellular metabolites (Hawkesford et al., 2012). In most plants, including *Chrysanthemum* spp., N deficiency results in growth restrictions, a dense rooting system, affected flower size and/or color as well as small, pale green leaves (Roorda Van Eysinga and Smilde, 1980). Hardly any information is available on foliar N sufficiency rates in *Chrysanthemum* spp., but 3% and 2.5% N on a dry weight basis have been reported as the lower limit to avoid stress (Lunt et al., 1964; Roorda Van Eysinga and Smilde, 1980). A foliar N content

Abbreviations: Ab, abaxial; Ad, adaxial; Chl, chlorophyll measured with Dualex; DAT, days after transplantation; DW, dry weight; EPhen, epidermal polyphenolics measured with Dualex; FW, fresh weight; LA, leaf area; LMA, leaf mass per area; N_A, area-based foliar nitrogen content; NBI, nitrogen balance index; NDVI, normalized difference vegetation index; N_M, mass-based foliar nitrogen content; Phen, polyphenolics; SPAD, chlorophyll measured by SPAD; VI, vegetation index

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over 5% is considered excessive. N uptake is known to occur continuously from planting onwards until the start of the flowering stage, thereafter uptake slows down (Lunt et al., 1964; MacDonald et al., 2013; Yoon et al., 2000). For reasons listed above, a proper N supply is essential for plant quality and the return on investment for farmers. This explains why oversupplying this nutrient is a recurring problem in high-value horticultural cropping systems, i.e. growers tend to consider N application as a safeguard for their income (Glass, 2003; Weinbaum et al., 1992). Unfortunately, this unintentional tendency to over-fertilize does not only lead to higher costs for growers, but also results in high environmental costs by leaching and runoff of NO_3^- and sometimes phosphates associated with N in fertilizers into ground and surface waters. Recently, more attention to optimize fertilization in outdoor ornamental production is given in order to reduce contamination of the environment due to leaching of nitrogen. Besides soil and plant analysis to estimate the need for additional N fertilization, non-destructive methods have been found useful.

As N is incorporated in chlorophyll, the foliar N content is positively correlated with the chlorophyll content (Evans et al., 2001; Peoples and Dalling, 1988). Therefore, proximal sensors based on the optical properties of chlorophyll can help plant growers match their N applications with the actual demand at a particular moment in time. These optical sensors have the advantage of being non-destructive, fast and convenient for in-field use. Most optical chlorophyll sensors send a red and infrared light pulse towards the plant tissues and subsequently measure the reflected and/or transmitted intensity of both wavelengths. Contrary to R light, IR light is barely absorbed by the chloroplast pigments. Hence, these measurements can give an indication of the amount of chlorophyll present in the leaves (Knipling, 1970; Li et al., 2014). Optical sensors can be divided into two main groups, operating either at individual leaf level or at canopy level. The first group, the chlorophyll meters or leaf-clips, have the advantage of being convenient, rapid and straightforward for in-field use at an affordable price. However, reflectance measurements of individual leaves might not be representative for the entire plant. Whereas chlorophyll meters measure one leaf at a time, the second group, the canopy reflectance meters, measure the light reflectance of a distinct area of the field, or even an entire field when tractor-mounted. These devices are often more expensive and results are more sensitive to environmental conditions (e.g. soil, weather, sensor-to-crop distance, seasonality) (Muñoz-Huerta et al., 2013).

The most renowned device to measure chlorophyll content at leaf level is the SPAD chlorophyll meter (SPAD-502, Konica Minolta, Osaka, Japan), emitting sequentially red (640 nm) and infrared (940 nm) light. The ratio of each transmitted and emitted wavelength is used as an indicator for the amount of chlorophyll in the leaf per unit of leaf area (Markwell et al., 1995). The SPAD meter was initially developed for measurements on rice (*Oryza sativa* L.) and was afterwards extensively studied in several other agricultural crops. More recently, the utility of the SPAD meter has been tested on ornamental plants such as poinsettia (*Euphorbia pulcherrima* L.) (Basyouni et al., 2015), several ornamental shrubs (Demotes-Mainard et al., 2008; Martín et al., 2007), carnation (*Dianthus chinensis* L.) (Basyouni et al., 2016) and gaillardia (*Gaillardia aristata* Pursh) (Dunn et al., 2015). Although calibration equations for multiple species were obtained in the past (Cerovic et al., 2012; Markwell et al., 1995), it was also observed that SPAD and other leaf clip readings can be affected by e.g. cultivar, leaf age and anatomical characteristics such as dry leaf mass per area (LMA) (Koyama et al., 2008; Louis et al., 2009; Neilsen et al., 1995; Turner and Jund, 1994; Xiong et al., 2015). Because optical crop sensors predict area-based pigment contents, changes in LMA can interfere with correlations between pigments and their mass based proxy (chlorophyll, N), and should for this reason be considered (Peng Shaobing et al., 1993). But, as LMA needs to be determined destructively, the advantages of non-destructive sensors are partly negated.

To improve the reliability of estimating leaf N content with non-destructive chlorophyll meters, other approaches have been proposed recently. Due to a mutual precursor, *L*-phenylalanine, N supply not only influences protein, and thus chlorophyll content, but also the polyphenolic (Phen) content. Hence, their synthesis is inversely correlated (Jones and Hartley, 1999) and therefore the Phen content can be of interest for the assessment of crop N status. The ratio of chlorophyll over Phen or the nitrogen balance index (NBI) is thus an indicator of N-deficiency due to different C/N-allocation under limited N supply (Cartelat et al., 2005). The use of a ratio of two area-based estimators should also erase the dependency on a varying LMA. Another approach considers the Phen content as a proxy for LMA as both parameters increase with irradiance (Cerovic et al., 2015, 2012; Poorter et al., 2009).

Goulas et al. (2004) described a portable leaf-clip instrument that can assess the concentration of epidermal Phen compounds (EPhen, mainly flavonoids) in leaves, using chlorophyll fluorescence: Dualex (FORCE-A, Orsay, France). Flavonoids have an UV light screening effect in the epidermis, reducing the deleterious effects of UV light reaching chlorophyll molecules. Measuring the emitted chlorophyll fluorescence after UV light illumination thus estimates its concentration. Dualex operates with an UV-A (375 nm) light source and a red reference beam (650 nm) (Goulas et al., 2004). The device's latest version, a Dualex Scientific, derives the chlorophyll content simultaneously from light transmission differences between two IR wavelengths (710 and 850 nm) (Cerovic et al., 2012). The one-sided NBI can therefore be calculated simultaneously, but should be recalculated afterwards using the total summed EPhen, as adaxial flavonoids tend to be more abundant than abaxial flavonoids in planophile plant leaves (Barnes et al., 2000; Kolb and Pfündel, 2005; Liakoura et al., 2003). The Dualex sensor has been tested in grapevines (*Vitis vinifera* L.) (Goulas et al., 2004), kale (*Brassica oleracea* L.) (Dunn et al., 2016), corn (*Zea mays* L.) (Tremblay et al., 2007), woody ornamentals (Demotes-Mainard et al., 2008) and several other crops.

At canopy level, reflectance meters can be used to measure the light reflectance of several wavelengths. The use of linear or non-linear combinations of wavelengths sensitive to estimate plant canopy parameters, such as leaf area index, biomass and ground cover, and insensitive to others (e.g. soil background, sun angle, atmospheric refraction) have led to numerous vegetation indices (VI) (Tucker, 1979). The Normalized Difference Vegetation Index (NDVI) is the most widely adopted VI for the estimation of biomass and nitrogen content (Goel, 1988). NDVI is calculated as the ratio of the reflected amount of (IR – R) and (IR + R) light and was first reported to be related with green biomass by Deering et al. (1974). A frequently used device to measure this index is the GreenSeeker NDVI-meter (GreenSeeker RT100, NTech Industries, Ukia, CA) (Basyouni et al., 2015; Ji et al., 2017; Kipp et al., 2014). This active sensor sends out light beams of 660 (R) and 770 nm (IR) to the canopy area and measures the reflection of emitted light of each wavelength captured by its detector. Other available sensors, e.g. CropScan, do not use an internal light source but measure the reflectance of sunlight instead and are called passive sensors (Li et al., 2014; Muñoz-Huerta et al., 2013). The GreenSeeker has been used in corn (*Zea mays* L.) (Freeman et al., 2007), poinsettia (*Euphorbia pulcherrima* L.) (Basyouni et al., 2015), cabbage (*Brassica oleracea* var. *capitata* L.) (Ji et al., 2017) and several other crops.

The objectives of this study are (1) to determine if SPAD and/or Dualex measurements can predict N content and thus N deficiency for two outdoor-grown pot *Chrysanthemum* cultivars 'Maya' and 'Orlando', (2) to examine the link between NDVI, biomass, foliar N content and plant N content. The validation of these two objectives is an essential step to determine the utility of non-destructive proximal sensors as an on-farm decision support system in *Chrysanthemum* cultivation.

2. Materials and methods

2.1. Sampling site, planting material and experimental design

Experimental work was conducted at PCS Ornamental Plant Research (PCS), Destelbergen, Belgium (51°04′18.3″N, 3°49′01.5″E). The soil had a fine sand texture and organic carbon content of 1.6%. Three experiments were carried out for three consecutive years (2016, 2017 and 2018).

Rooted cuttings of *C. morifolium*, 'Maya' and 'Orlando' (cushion phenotype), were obtained from a commercial propagator (Dataflor, Zonnebeke, Belgium) and transplanted into 3.5 l pots with commercial potting mixture (80% sphagnum peat;20% clay) mixed with 1.3 and 1 kg m^{-3} of 14N-16P-18 K fertilizer (PG Mix®, quick release fertilizer) for 2016 and both 2017 and 2018, respectively. In 2016, 2 kg m^{-3} of 17N-10P-11 K controlled release fertilizer (Osmocote® Pro, 5/6 month product) was mixed within the substrate as well. In 2017, 0.23, 0.69 and 1.14 kg m^{-3} 19N-6P-20 K fertilizer (Kristalon® Blue, fast dissolving water soluble product) dissolved in rain water was supplied instead (5 times one application $week^{-1}$) in order to create different substrate N concentrations from the beginning of the experiment onwards. In 2018, Kristalon® Blue was replaced by a singular application of 0.43, 0.86 and 1.29 kg m^{-3} granulated 22N-5P-11 K fertilizer (Osmoform®, 6 weeks product). No corrections for P and K were applied to the other treatments.

After potting, the plants were placed outdoors on a container field mid-May and transported to the open field after 4 weeks of growth in 2016 (exact dates in Table A1, see Appendix A). This procedure was similar for 'Maya' and was delayed by 2 weeks for 'Orlando' in 2017 and 2018. Measurement and sampling days are referred to as days after transplantation (DAT) to the open field. All experimental plants were surrounded by border plants.

Planting distance was 0.45 m between rows and 0.90 m within rows. The 2016 sampling site consisted of 9 plots of 8 m x 4.15 m (298.8 m^2), each 4 rows of 8 plants per cultivar. This area was doubled in the second and third year (597.6 m^2). Top soil (0–30 cm) in June contained 12 kg ha^{-1} mineral N in 2016, 27 kg ha^{-1} in 2017 and 13 kg ha^{-1} in 2018. For each cultivar, a randomized block design with three replicates for each N level was used. Rates were selected based on previous observations for *Chrysanthemum* response on N and adapted after soil analysis prior to starting. The following nitrogen soil dressing treatments were applied to provide plants with N levels ranging from deficient to excessive for the three consecutive years: 0 – 77–153 kg N ha^{-1} in 3 applications (calcium ammonium nitrate, 27% N), 0 – 53–107 kg N ha^{-1} in 4 applications (Tropicote®, 15.5% N) and 0 – 50–75 kg N ha^{-1} in 3 applications (Tropicote®, 15.5% N) with intervals of 1 to 4 weeks, depending on weather forecasts (Table 1, timing in Table A1, see Appendix A). Before transplantation to the field in 2016, each plot was fertilized with 2 kg Patentkali (30% K_2O, 10% MgO and 42.5% SO_3). In 2018 an additional dose of 457 g $plot^{-1}$ was given in July.

Table 1
Overview of applied N fertilizer rates (kg N ha^{-1}), mixed with the substrate or applied as soil dressing after transplantation to the field. N0: deficiency treatment, N1: standard treatment, N2: high N rate treatment.

		Treatment								
		2016			2017			2018		
Application method	Fertilizer	N0	N1	N2	N0	N1	N2	N0	N1	N2
in pots	*PG Mix®*	13	13	13	10	10	10	10	10	10
	Osmocote® Pro	24	24	24	0	0	0	0	0	0
	Kristalon® Blue	0	0	0	3	9	15	0	0	0
	Osmoform®	0	0	0	0	0	0	7	13	20
as soil dressing	*CAN*	0	77	153	0	0	0	0	0	0
	Tropicote®	0	0	0	0	53	107	0	50	75
Total N applied		**37**	**114**	**190**	**13**	**72**	**132**	**17**	**73**	**105**

Abbreviations: CAN, Calcium Ammonium Nitrate.

Table 2
Average precipitation (mm) and air temperature (°C) during *Chrysanthemum* growing months at the experimental site and 30-year average data from RMI Belgium, Ukkel (1981–2010).

	Precipitation (mm)				Air temperature (°C)			
	P_{2016}	P_{2017}	P_{2018}	P30-yr avg.	T_{2016}	T_{2017}	T_{2018}	T30-yr avg.
May	115.6	24.2	28.8	66.5	14.4	15.6	16.2	13.6
June	98	22.4	25.2	71.8	16.8	19.5	18	16.2
July	27	68.4	3.8	73.5	19.4	18.8	21.8	18.4
August	44.2	71.0	69.8	79.3	18.9	18.1	19	18
September	16	71.6	43.8	68.9	17.6	14	14.8	14.9
October	53.4	41.0	30.8	74.5	10.1	13.4	12.2	11.1

Abbreviations: RMI, Royal Meteorological Institute of Belgium; P, Precipitation; T, Temperature; 30-yr avg., 30-year average.

The growing seasons were characterized by contrasting weather conditions (Table 2). In May and June precipitation was respectively high for 2016 and exceptionally low for 2017. July – September were relatively dry in 2016 and average in 2017. July 2018 was extreme dry and warm. October was relatively dry in all years. Overhead sprinkler irrigation was installed all three years to prevent excessive drought stress. Weed and pest management were according to standard horticultural practices.

2.2. Plant quality measurements

Plant height (from the edge of the pot to the apical shoot, bud or flower) and diameter (average of two perpendicular measurements) were measured at 2 to 3-week intervals, this for five preselected plants per plot. At the same time aboveground plant biomass of one representative plant per plot in 2016 and 2017 and two in 2018 (= three or six plants per N treatment) was measured, dried at 60 °C for 48 h for mass based N content determination by dry combustion analysis using an elemental analyzer (Vario MAX CNS, Elementar, Germany).

2.3. Optical measurements at leaf level

A total of 4410 leaves were sampled during the experiment. Approximately every two to three weeks, 10 leaves per plot (= 30 leaves per N treatment) for the first two years and 15 leaves per plot (= 45 leaves per N treatment) in 2018 per cultivar were selected from the middle to upper area of the plants. Only fully expanded sun-exposed leaves were measured (Fig. 1). A SPAD-502 chlorophyll meter (Konica

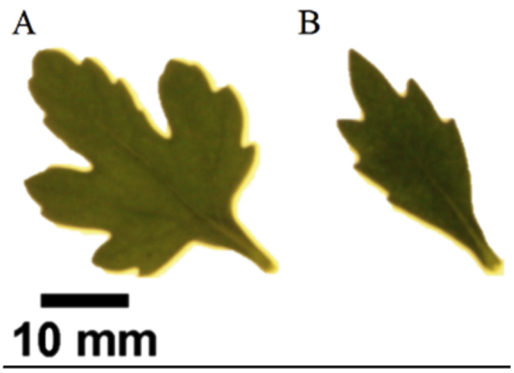

Fig. 1. Adaxial sides of the leaves of *Chrysanthemum morifolium* 'Maya' (A) and 'Orlando (B).

Minolta, Osaka, Japan) was used in 2016 and 2017 years to estimate area-based chlorophyll content. Per leaf, three different readings were taken for 'Maya' and two different readings for 'Orlando' (due to smaller leaf size) at the adaxial side of the leaf, avoiding the mid-rib. In 2017 and 2018 a Dualex Scientific meter (FORCE-A, Orsay, France) was used additionally to estimate area-based chlorophyll content (Chl) and epidermal polyphenols (EPhen). Two measurements were taken on both adaxial and abaxial sides of the same leaves used for SPAD and averaged to estimate Chl. For total EPhen, adaxial and abaxial EPhen was summed. The nitrogen balance index (NBI) was calculated as the ratio between mean Chl and total EPhen ($NBI = Chl \times EPhen^{-1}$).

Digital images of leaves were analyzed by ImageJ software (version 1.51j8) to obtain the leaf area (LA). Leaf mass per area (LMA, g m^{-2}), was calculated as $LMA = DW \times LA^{-1}$. Leaf dry weight (DW, g) was determined by weighing the leaves after drying at 60 °C for 48 h.

For foliar nitrogen analysis the dried leaves were pooled per plot, grinded and stored until further analysis. Mass based N content (N_M) of the samples was determined by dry Dumas combustion analysis using an elemental analyzer (Vario MAX CNS, Elementar, Germany). The lower sufficiency limit of 2.75% N_M was calculated as the average value of the lower sufficiency values found in literature, respectively 3% and 2.5% (Lunt et al., 1964; Roorda Van Eysinga and Smilde, 1980). By multiplying N_M with LMA, area-based N content (N_A) was obtained ($N_A = N_M \times LMA$).

2.4. Optical measurements at canopy level

All plant rows from each plot were scanned at the same frequency as measurements at leaf level with a GreenSeeker to obtain NDVI (GreenSeeker RT100, NTech Industries, Ukia, CA). Due to a technical defect at the end of the season in 2018, the last sampled plants could not be scanned. The sensor-canopy distance was kept between 80 and 100 cm to enable stable sensor output (Kipp et al., 2014). Measurements were taken within 2 h from local solar noon to minimize influences by solar irradiation differences (Beneduzzi et al., 2017). In 2016, the harvested plants did introduce gaps in the rows, which were measured along with the GreenSeeker and might reduce average NDVI of that row. This was avoided in the later years.

2.5. Statistical analysis

Statistical analysis was carried out with R version 3.4.1 software (R Core Team, 2017). Data are presented as means ± SE. The effect of N treatments on the studied plant variables was assessed using analysis of variance (ANOVA) followed by Tukey's honest significant difference (HSD) post hoc test ($p \leq 0.05$). The normal distribution and the homoscedasticity of variance assumptions were checked using Shapiro-Wilk and Bartlett test, respectively. In some cases, when normality or homoscedasticity was severely violated, the data were ln-transformed. Pearson's correlation coefficients (r) and regression techniques were performed to analyze the correlation between optical sensor measurements and N content in leaves and plants, using all the sampling dates and replicates for the cultivars considered. Analyses of covariance (ANCOVA) were conducted to test cultivar and growing season dependency on the relation between EPhen versus N_M and on N uptake versus FW.

3. Results

3.1. Effects of fertilization levels on plant quality (height, diameter and biomass) and N uptake

No significant differences in plant height and diameter were observed during the growing season (data not shown), nor at harvest time (Table 3). However, the zero fertilizer treatment tended to result in a smaller diameter. When comparing the three seasons, plant biomass and height were noticeably smaller in 2017 and 2018 compared with the first growing season.

At the end of the growing season smallest aboveground biomass was observed for all N0 treatments, but this was only significant for 'Maya' in 2016 (Fig. 2A). During the growing season, biomass differed significantly between fertilization levels for 'Maya' in 2017 at 79 DAT (N2 > N0) (Fig. 2B). This was also the case for 'Orlando' in 2016 at 69 DAT (N1 > N0 and N2 > N0) and at 79 DAT (N2 > N0) (Fig. 2D). At 51, 68 and 77 DAT, N1 and N2 resulted in a higher biomass compared to N0 for 'Orlando' in 2017 (Fig. 2E). No significant differences in aboveground biomass were noted in 2018 (Fig. 2C and 2 F). Total aboveground N uptake of 'Maya' was significantly higher for N1 and N2 compared to N0 in all three years. For 'Orlando', N2 showed a higher N uptake compared to N0 in 2017, while in 2016 and 2018 no differences were found (Table 3).

3.2. Seasonal changes in leaf N content, LMA and optical leaf measurements during treatments

Leaf mass-based N content (N_M) varied widely within each growing season. In 2016, N_M of 'Maya' first decreased from almost 3% to ± 2.5% at 28 DAT and then increased to a maximum of almost 4% at 83 DAT (Fig. 3A). At the end of the growing season N_M dropped again. In 2017 a similar pattern was observed without a drop in N content towards the end of the growing season (Fig. 3B). In 2018, early measurements revealed a high N_M at the very beginning of the growing season (Fig. 3C). Significant differences were found at 83 and 112 DAT in 2016, at 50, 65, 77 and 103 DAT in 2017 and at 2, 13, 77 and 99 DAT in 2018. N_M of 'Maya' in 2016 dropped under the sufficiency limit of 2.75% N twice for N0 and N1 and once for N2. This occurred in 2017 for all treatments at the second sampling moment and for N0 at three later sampling moments as well. In 2018 this occurred twice for N0 only and once for all three treatments.

Differences between treatments were minimal for 'Orlando' in 2016, only at the start (13 DAT), N2 had a significant higher N_M (Fig. 3D). In 2017, N_M of N0 treatment was significantly lower from the early start (N0 < N2) until 37 DAT (N0 < N1,2) and then increased quickly (Fig. 3E). Significant differences between the 'Orlando' treatments only occurred twice in 2018, at 66 and 88 DAT (Fig. 3F). N_M of 'Orlando' was in 2016 only deficient at the first sampling moment for N0 and N1. In 2017, the N0 treatment resulted in foliar N contents below 2.75% N in the first half of the growing season. In 2018 the foliar N content of 'Orlando' was always above the postulated threshold.

Leaf mass per area (LMA) was determined in 2017 and 2018. For 'Maya 2017, LMA increased shortly after transplantation to the field, decreased immediately and stagnated around 80 g m^{-2} (Fig. B1 A, see Appendix B). Leaf area decreased more quickly than leaf mass, leading to a high LMA peak at 21 DAT. A similar pattern was observed in 2018 (Fig. B1 B, see Appendix B). In contrast, 'Orlando' maintained a fairly constant LMA in 2017 (around 70 g m^{-2}) during the entire growing season, with exception of the N0, having a significantly higher LMA compared to the other treatments at 10 and 37 DAT (Fig. B1 C, see Appendix B). For 'Orlando' 2018, LMA was generally lower compared to 2017 while no significant differences were present between treatments (Fig. B1 D, see Appendix B). For 'Maya', leaf area-based nitrogen content (N_A) results in less significant differences compared to N_M in 2017 while the opposite was true for 'Orlando (Fig. 3B and E). It can be noticed that the difference between N_A of the fertilization treatments is much smaller at 37 DAT compared with N_M (Fig. 3E), at this moment LMA of N0 is significantly bigger as well (Fig. B1 C, see Appendix B).

In 2016, the SPAD meter could only detect different chlorophyll contents between the fertilization levels at one time point for both cultivars (data not shown). In 2017, no differences between treatments were found in SPAD for 'Maya' and once for Chl at 79 DAT (Fig. 4A). In 2018, Chl differed at 56 and 99 DAT (Fig. 4B). For 'Orlando', differences in SPAD occurred for all but one moment in 2017 (91 DAT).

Table 3
Final plant quality measurements and nitrogen uptake of two *Chrysanthemum* cvs. 'Maya' and 'Orlando'. Values are means ± SE. Values labelled by different letters significantly differ at P ≤ 0.05 (Tukey's HSD-test).

Cultivar	Year	DAT	N treatment	Applied N (kg ha^{-1})	Height (cm)	Diameter (cm)	Aboveground biomass (g plant^{-1})	Aboveground N uptake (kg ha-1)
Maya	2016	113	N0	37	33.4 ± 1.2a	57.3 ± 1.9a	290.6 ± 15.5b	102.8 ± 11.6b
			N1	114	34.8 ± 0.7a	59.3 ± 1.6a	373.1 ± 8.8a	146.2 ± 9.9a
			N2	190	32.7 ± 0.4a	60.8 ± 1.2a	360.2 ± 15.5a	154.3 ± 4.6a
	2017	104	N0	13	30.9 ± 0.2a	55.9 ± 1.2a	203.9 ± 48.9a	55.6 ± 9.1b
			N1	72	30.9 ± 0.6a	57.9 ± 0.7a	303.8 ± 27.7a	106.4 ± 7.7a
			N2	132	30.8 ± 0.8a	58.8 ± 1.2a	321.8 ± 33.3a	124.6 ± 15.8a
	2018	99	N0	17	27.3 ± 1.5a	50.5 ± 1.2a	186.4 ± 9.8a	57.8 ± 4.3b
			N1	73	30.7 ± 1.2a	58.3 ± 2.1a	261.9 ± 24.1a	102.1 ± 13.7a
			N2	105	32.2 ± 1.2a	58.4 ± 2.9a	263 ± 22.2a	102.7 ± 12.5a
Orlando	2016	126	N0	37	31.7 ± 0.6a	51.2 ± 0.7a	289.5 ± 33.1a	122.7 ± 9.6a
			N1	114	32.8 ± 0.7a	53.9 ± 0.4a	333.5 ± 17.2a	137.6 ± 7.3a
			N2	190	31.9 ± 0.9a	52.8 ± 1.4a	332.4 ± 25.6a	165.7 ± 14.4a
	2017	106	N0	13	25.8 ± 0.5a	41.7 ± 0.7a	172.3 ± 24.6a	60.4 ± 9.1b
			N1	72	28.4 ± 1.3a	46.3 ± 1.1a	206.2 ± 23.1a	79.6 ± 6.1ab
			N2	132	27.3 ± 1.7a	45.3 ± 1.5a	231.5 ± 2.6a	97.3 ± 0.9a
	2018	109	N0	17	25.3 ± 0.5a	45.4 ± 0.5a	184.9 ± 7.6a	71.8 ± 4.6a
			N1	73	26.5 ± 0.9a	47.3 ± 2.0a	195.1 ± 19.0a	86.1 ± 3.8a
			N2	105	27.7 ± 1.1a	49.3 ± 1.1a	208.3 ± 14.3a	90.8 ± 5.8a

Abbreviations: DAT, Days After Transplantation.

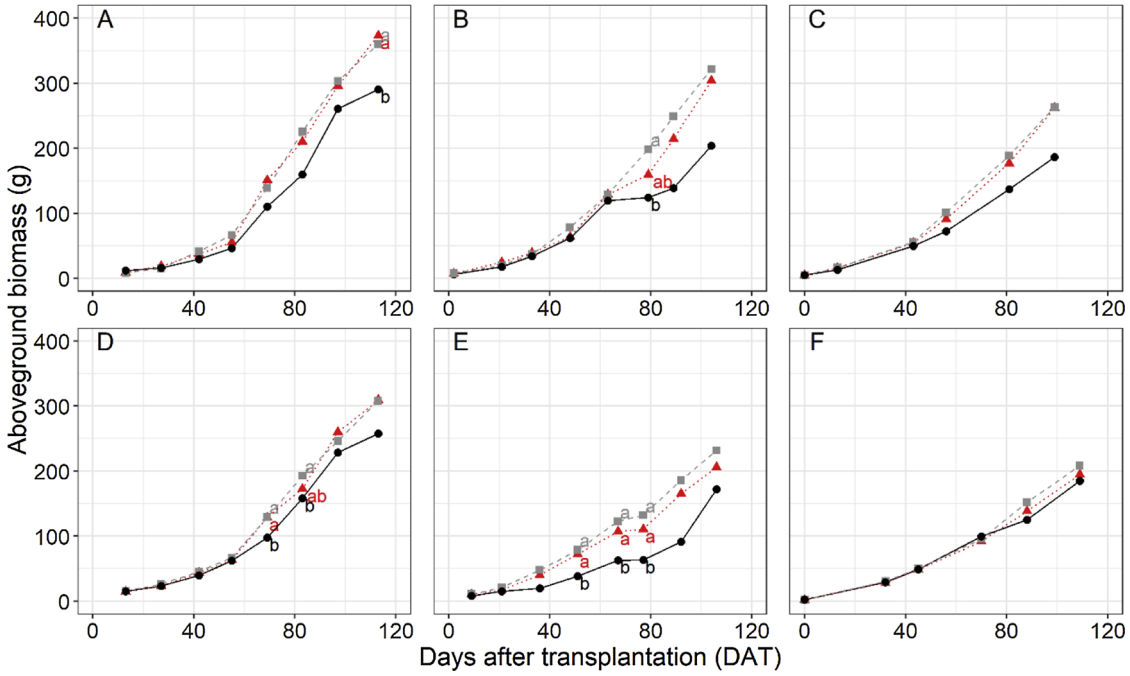

Fig. 2. Biomass increase in each cultivar at each experimental year (A: 'Maya' 2016, B: 'Maya' 2017, C: 'Maya' 2018, D: 'Orlando' 2016, E: 'Orlando' 2017, F: 'Orlando' 2018). Full black, dotted red and dashed grey lines represent N0, N1 and N2 treatments respectively. Each point is the mean of three replicates. Means labelled by different letters did significantly differ at P ≤ 0.05. If no letters are present, no significant differences were present at that time point.

However, SPAD values did not differ among N1 and N2 at any point in time (Fig. 4C). Similar results were found for Chl. Significant differences in Chl of 'Orlando' were absent in 2018 (Fig. 4D). The epidermal polyphenolics content (EPhen) of N0 was significantly higher than that of N2 from 50 DAT onwards until the end of the crop cycle (with the exception of 91 DAT) for 'Maya' in 2017 (Fig. 4A). No differences could be detected in 2018 (Fig. 4B). For 'Orlando', EPhen values differed at 10, 28 and 37 DAT in 2017 (Fig. 4C) and at 88 DAT in 2018 (Fig. 4D). NBI was generally able to detect a higher number of differences between treatments for 'Maya' and 'Orlando', with the exception of 'Orlando' in 2018 (Fig. 4).

3.3. Changes in plant N content and optical plant measurements (NDVI) during treatments

The plant N content differed greatly over the growing seasons and between the cultivars and was mostly lower than the N_M content in the leaves (Figs. 3 and 5 A–F). Concentrations in 2017 were generally lower compared to concentrations in 2016 and 2018 for both cultivars. Except for N0 of 'Orlando' in 2017, the plant N concentration fell gradually during the growing season with some fluctuations in between (Fig. 5A–F). In 2016, the three different nitrogen treatments did not result in significant differences in N content, except for 'Orlando' at 126 DAT (Fig. 5D). In 2017 however, the nitrogen content of N0 of 'Maya' was lower at 89 and 104 DAT (Fig. 5B). This was also the case at all but two sampling moments for 'Orlando' (Fig. 5E). In 2018, treatments only

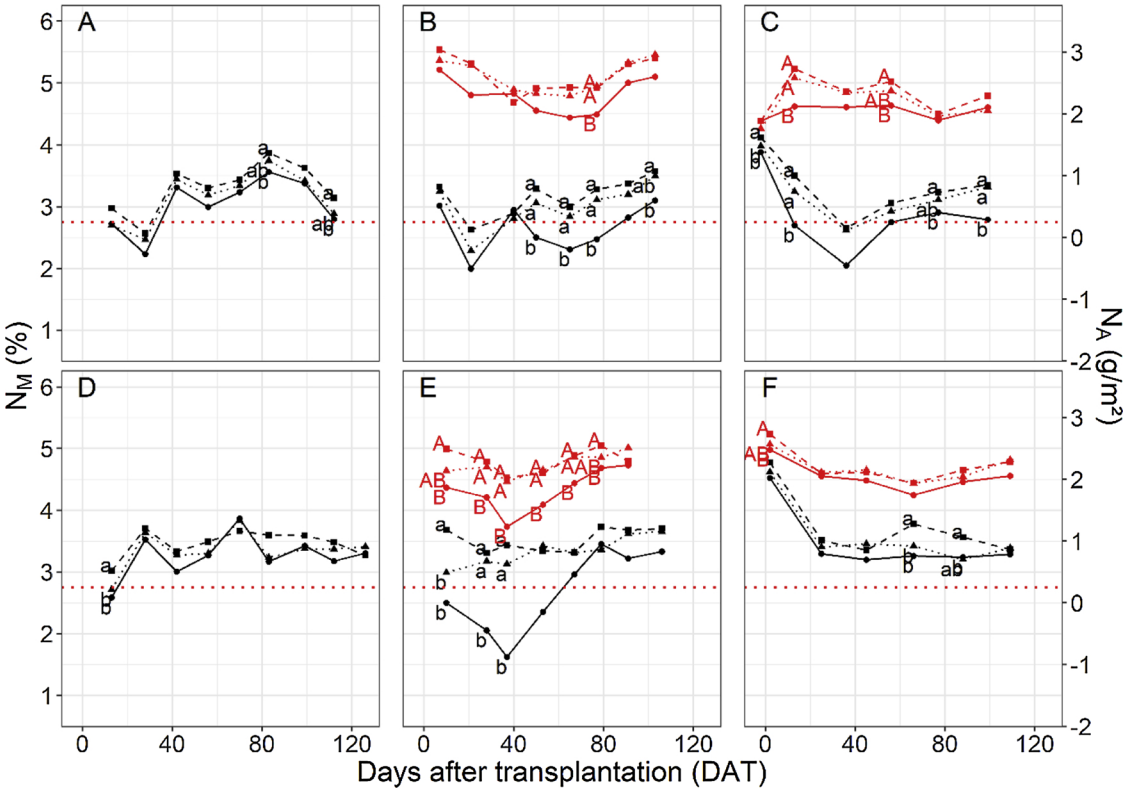

Fig. 3. Changes of N content of young full grown leaves of the two *Chrysanthemum* cultivars (A: 'Maya' 2016, B: 'Maya' 2017, C: 'Maya' 2018, D: 'Orlando' 2016, E: 'Orlando' 2017, F: 'Orlando' 2018). Black lines represent the N content in terms of dry mass (N_M). Red lines represent the N content in terms of leaf area (N_A). Full, dotted and dashed lines represent N0, N1 and N2 treatments respectively. The dotted horizontal line at 2.75% N_M represents the lower sufficiency limit for *Chrysanthemum*. Means labelled by different letters (small letters for N_M, capital letters for N_A) did significantly differ at $P \leq 0.05$. If no letters are present, no significant differences were present at that time point. Each point is the mean of 3 replicates of 10 leaves in 2016 and 2017 and of 15 leaves in 2018.

resulted in significant differences for 'Maya' at the very beginning of the growing season, and at the end as well (Fig. 5.C). There were little differences in N concentration between N1 and N2 treatments for both cultivars and all years.

NDVI increased for all treatments throughout the measuring period (Fig. 5G-L). In 2016, both cultivars' NDVI show an S-shaped curve. The NDVI increase of 'Maya' levels off at the end of the measuring period in 2016 and 2017, which was not the case in 2018. NDVI at the end of the growing season differs approximately 0.1 units between 2016 at the one hand and the two later years on the other hand (± 0.7 in 2016 vs. ± 0.8 in 2017 and 2018) (Fig. 5G-I). This difference is also present for 'Orlando', but not as distinctive as for 'Maya (± 0.68 in 2016 vs. ± 0.73 in 2017 and 2018) (Fig. 5J-L). Although the N0 treatment showed the lowest NDVI at any time, GreenSeeker was only able to detect significant differences between treatments at 55 DAT in 2016, 63 and 89 DAT in 2017 and at 56 and 81 DAT in 2018 for 'Maya' and at 39 and 51 DAT for 'Orlando' in 2017 (Fig. 5G-L).

3.4. Correlations between Chl, EPhen, NBI and N content in leaves

All sampling days were considered to identify useful parameters to predict foliar N content. Correlations between LMA, optically measured parameters and foliar N (mass- and area-based) were investigated. Correlations between SPAD and N_M were absent in 2016 for both cultivars (data not shown). An overview of the most important correlations for 2017 and 2018 are given in Table 4.

In 2017, SPAD was recurrently investigated but LMA and Dualex measurements were included as well. In 2018, SPAD measurements were no longer performed. A significant but only moderate correlation for SPAD and N_M (Pearson's r = 0.56) was found for 'Orlando'. When comparing with mass-based SPAD (SPAD/LMA) instead, the correlation became significant for 'Maya' as well (Table 4). Also mass-based Chl (Chl/LMA) correlated better with N_M compared with the original sensor value. However, Fig. 6A shows clearly that R^2 are only moderate and that the curves tend to saturate quickly. Similar findings were found for Chl measured with Dualex in 2017 (Fig. 6 B). In 2018 however, the Pearson correlation between Chl and N_M is negative for both cultivars (Fig. 6C). When the very first sampling moment, characterized by a low LMA and a high N_M, is excluded from the dataset (DAT = -2 for 'Maya' and 2 for 'Orlando'), Pearson's r is positive but not significant (r = 0.28, p = 0.06 for 'Maya' and r = 0.11, p = 0.47 for 'Orlando'). Since NBI is the Chl:EPhen-ratio, the first measuring moment also affects the correlation with NBI in 2018 (Table 4).

Strongest correlations for both years and cultivars were found between foliar N_M content and EPhen (Table 4). Analysis of covariance (ANCOVA) showed that the slopes of the regression equation were different for each cultivar per year ($P \leq 0.05$). For 'Maya' 2017, a polynomial second order quadratic model (EPhen = $5.4 - 1.5N_M + 018\ N_M^2$, $R^2 = 0.72$) fitted the relationship between N_M and EPhen better than a linear model ($P \leq 0.05$), indicating saturation at high N_M levels (Fig. 7A). This was also the case in 2018 (EPhen = $3.7 - 0.073\ N_M - 0.12\ N_M^2$, $R^2 = 0.86$) (Fig. 7B). This was not observed for 'Orlando' 2017, where a linear model was sufficient to describe the relation between N_M and EPhen (EPhen = $4.6 - 0.57\ N_M$, $R^2 = 0.76$) (Fig. 7D). A linear model was also adequate for this cultivar in 2018 (EPhen = $4.0 - 0.37\ N_M$, $R^2 = 0.74$) (Fig. 7E).

The correlation of N_M between total, adaxial and abaxial EPhen is slightly different and adaxial EPhen are higher than abaxial EPhen (Fig. 7). Also one-sided EPhen measurements, especially that of abaxial EPhen, are equally well or even better correlated with N_M. The correlation between abaxial EPhen and N_M is cultivar dependent as well and is given by the following equations in 2017 for 'Maya' and 'Orlando'

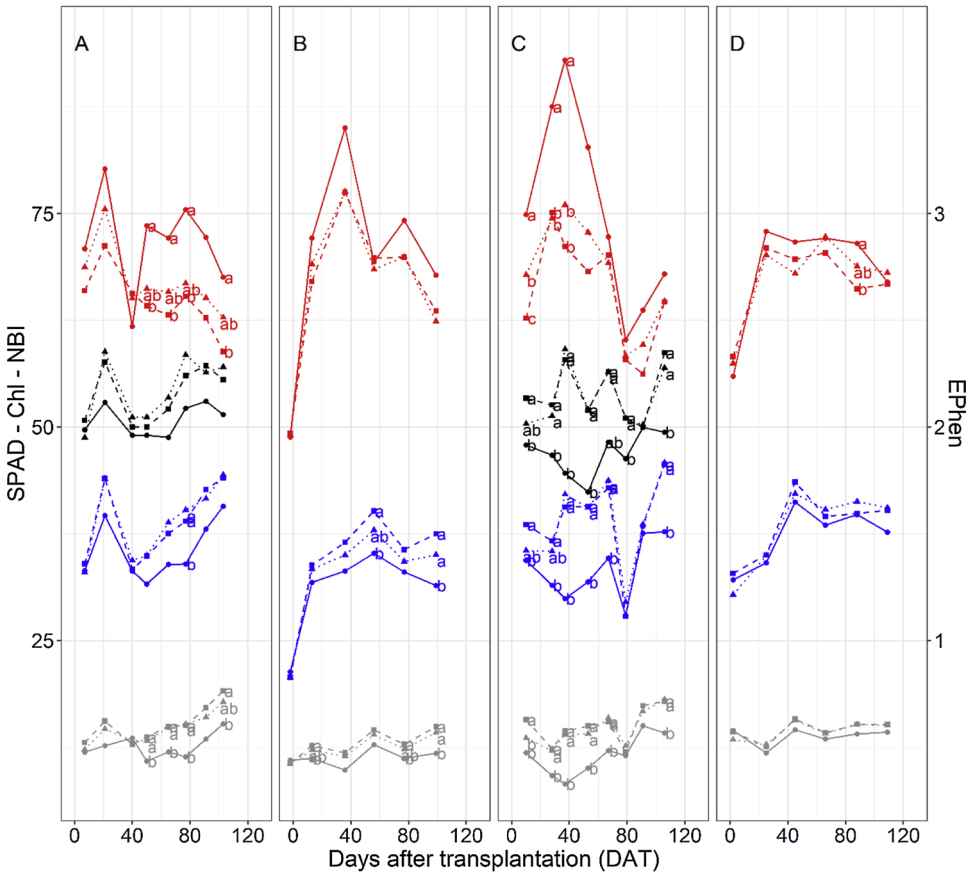

Fig. 4. Changes in optical leaf parameters of young full grown leaves of the two *Chrysanthemum* cultivars (A: 'Maya' 2017, B: 'Maya' 2018, C: 'Orlando' 2017, D: 'Orlando' 2018). Black lines represent area based chlorophyll content measured with SPAD, blue lines chlorophyll content measured with Dualex (Chl), red lines the epidermal polyphenolics (EPhen) measured with Dualex and grey lines NBI (nitrogen balance index = Chl:EPhen-ratio). Full, dotted and dashed lines represent N0, N1 and N2 treatments respectively. Means labelled by different letters did significantly differ at $P \leq 0.05$. If no letters are present, no significant differences were present at that time point. Each point is the mean of 3 replicates of 10 leaves in 2016 and 2017 and of 15 leaves in 2018.

respectively: $EPhen_{ab} = 2.3 - 0.61\ N_M + 0.064\ N_M^{\ 2}$ ($R^2 = 0.79$) and $EPhen_{ab} = 2.1 - 0.31\ N_M$ ($R^2 = 0.74$). In 2018 the correlation equations are: $EPhen_{ab} = 2.1 - 0.37\ N_M$ ($R^2 = 0.84$) and $EPhen_{ab} = 1.9 - 0.25\ N_M$ ($R^2 = 0.74$). Fig. 7C and F show the regression for 'Maya' and 'Orlando' when both years are considered. ANCOVA pointed out that 'year' had a significant effect on the regression equation ($EPhen_{ab}$ versus N_M) for 'Maya' but not for 'Orlando', thus common equations for both years can be used for this cultivar: $EPhen_{ab} = 2.2 - 0.42\ N_M + 0.022\ N_M^{\ 2}$ ($R^2 = 0.74$).

3.5. Correlations between NDVI, height, diameter, biomass and N content/uptake in plants

According to the Pearson correlation tests N uptake and FW, DW, height and plant diameter closely correlated for both cultivars and experimental years separately (Table B1, see Appendix B). Combining the data of the three years and both cultivars also resulted in very high overall correlations, despite ANCOVA analysis between ln transformed N uptake and FW showed that both cultivars and years had slightly different regression equations ($P < 0.001$) (Fig. 8).

All sampling days were considered to determine whether NDVI was correlated with plant parameters throughout the growing season (Table 5). NDVI correlated very well with biomass (fresh and dry weight) for both cultivars and all three years. NDVI correlated in most cases even better with non-destructive and easy to measure parameters such as height and diameter. Weak negative correlations were found between NDVI and plant N% for 'Maya' in all three years and for 'Orlando' in 2016 and 2018. A linear relation between NDVI and FW (log transformed) is presented (Fig. 9). The regression equation for 'Maya' differs between 2016 at one hand and 2017 and 2018 at the other hand. A combined equation for the last two years is: $\log(FW) = 1.25 + 2.14$ NDVI. For 'Orlando' the regression is only valid on annual base.

4. Discussion

4.1. Variability in plant quality and N concentration

The visual plant quality of pot *Chrysanthemum* is determined by plant diameter and the presence of a compact bushy hemispherical phenotype. This plant quality is associated with applied nitrogen levels as we also found in this research. Lower nitrogen supply in 2016 resulted in a reduced plant quality for 'Maya' if no supplemental nitrogen was given (N0) and only N from soil mineralization was available when roots penetrated into the soil (Fig. A1, see Appendix A). Although not often significant, diameter, biomass and aboveground N uptake were also consistently smaller for the N0 treatments for all years and cultivars. Overhead sprinkler irrigation was installed, but it is assumable that the dry weather conditions at the start of the second experimental year caused a reduced root growth resulting in a relatively lower plant height, diameter and biomass and N uptake later that year. This was also the case in 2018, especially in July shortly after the plants were transplanted to the field and when there was almost no natural rainfall in combination with very high temperatures.

N fertilization resulted in an increase in N_M compared to the N0 treatment though seasonal changes were present as well. The foliar N_M sufficiency value of 2.75% in *Chrysanthemum* spp. (Lunt et al., 1964; Roorda Van Eysinga and Smilde, 1980) was not always reached. Especially in the first part of the growing season of 2017, N_M of N0 averaged below the lower limit. The limited rainfall in May-June probably resulted in lower N mineralization and availability (Fig. 3); when weather conditions were more favorable, the gap became smaller. Despite of the first part of the growing season of 2018 being characterized by similar weather conditions, this large gap was not observed. Although N_M was generally only a few times below the limit of 2.75% for N0, this treatment tended to have the smallest diameter and

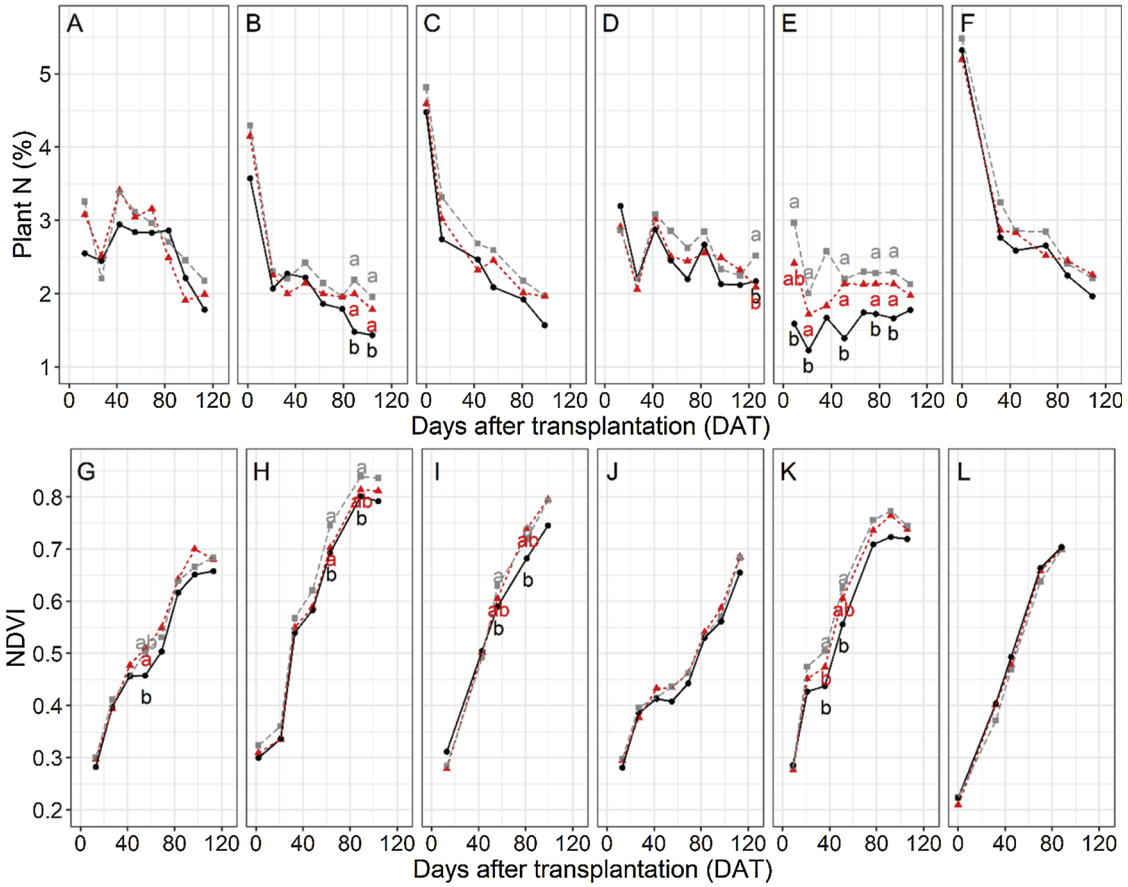

Fig. 5. Seasonal changes in plant N content (%) and NDVI of the two *Chrysanthemum* cultivars (A,G: 'Maya' 2016, B,H: 'Maya' 2017, C,I: 'Maya' 2018, D,J: 'Orlando' 2016, E,K: 'Orlando' 2017, F,L: 'Orlando' 2018). Full, dotted and dashed lines represent N0, N1 and N2 treatments respectively. Each data point is the mean of 3 replicates of one plant in 2016 and 2017 and two pooled plants in 2018. Means labelled by different letters did significantly differ at $P \leq 0.05$. If no letters are present, no significant differences were present at that time point.

biomass. This implies that the suggested foliar N sufficiency threshold of 2.75% is rather low for recent cultivars. Low N_M clearly coincides with high LMA values (thicker leaves) (Fig. 3 and Fig. B1, see Appendix B). This can be explained by the fact that when the LMA is high, the amount of nitrogen is diluted in a larger amount of leaf material (Jones and Hartley, 1999; Meyer et al., 2006).

Table 4
Pearson correlation coefficient (r) of optical parameters (SPAD, Chl, EPhen, NBI) vs. destructively measured leaf parameters (N_M, N_A) in two *Chrysanthemum* cvs. 'Maya' and 'Orlando' for 2017 (n° of data point between brackets).

	Maya 2017	Maya 2018	Orlando 2017	Orlando 2018	Overall
SPAD vs. N_M	0.2 (72)ns	–	0.56 (72)***	–	0.22 (297)***
SPAD vs. N_A	0.52 (72)***	–	0.53 (62)***	–	0.56 (135)***
SPAD/LMA vs. N_M	0.62 (72)***	–	0.72 (62)***	–	0.66 (135)***
Chl vs. N_M	0.24 (72)*	−0.58 (54)***	0.44 (72)***	−0.6 (54)***	−0.01 (252)ns
Chl vs. N_A	0.56 (72)***	0.6 (54)***	0.33 (62)*	−0.4 (54)**	0.36 (243)***
Chl/LMA vs. N_M	0.66 (72)***	0.43 (54)**	0.58 (62)***	−0.24 (54)ns	0.53 (243)***
EPhen vs. N_M	−0.82 (72)***	−0.92 (54)***	−0.86 (72)***	−0.85 (54)***	−0.77 (252)***
EPhen vs. N_A	−0.35 (72)**	0.34 (54)*	−0.8 (62)***	−0.73 (54)***	−0.34 (243)***
EPhen/LMA vs. N_M	0 (72)ns	0.47 (54)**	−0.38 (62)*	−0.3 (54)*	−0.23 (243)***
NBI vs. N_M	0.6 (72)***	0.1 (54)ns	0.77 (72)***	0.03 (54)ns	0.45 (252)***
NBI vs. N_A	0.6 (72)***	0.48 (54)**	0.7 (62)***	0.16 (54)ns	0.54 (243)***
Chl vs. SPAD	0.86 (72)***	–	0.79 (72)***	–	–
LMA vs. N_M	−0.53 (72)***	−0.85 (54)***	−0.7 (62)***	−0.46 (54)**	−0.66 (243)***

Abbreviations: SPAD, chlorophyll measured with a SPAD meter; N_M, mass-based foliar N content; N_A, area-based nitrogen content; LMA, leaf mass per area; Chl, chlorophyll measured with Dualex; EPhen, epidermal polyphenol content measured with Dualex; NBI, nitrogen balance index.
ns = non-significant.
* = significant at $P \leq 0.05$.
** = significant at $P \leq 0.01$.
*** = significant at $P \leq 0.001$.

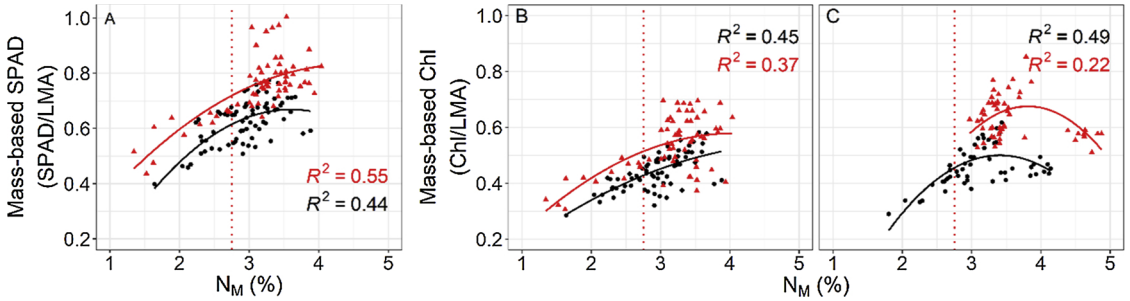

Fig. 6. Relation between the mass-based N content (N_M) and mass-based SPAD (A) and mass-based Chl measured with Dualex in 2017 (B) and 2018 (C) for 'Maya' (red triangles) and 'Orlando' (black dots). The dotted vertical line at 2.75% N_M represents the lower sufficiency limit for *Chrysanthemum*. Each point is the mean of 3 replicates of 10 leaves in 2016 and 2017 and of 15 leaves in 2018.

4.2. Relationships between chlorophyll meter readings, Greenseeker and nitrogen status

In both measuring years, SPAD was hardly able to distinguish between fertilization levels, except for 'Orlando' in 2017 (Fig.4). This was not unexpected, given the limited differences in N_M between the different treatments in 2016 (Fig. 3A and D) and for 'Maya' in 2017 (Fig. 3A). As LMA was also determined in 2017, we calculated N_A. This parameters explains the lack of distinction by SPAD, as relative smaller differences in N_A existed compared with N_M on the consecutive measuring dates and SPAD measurements are stronger for area-based correlations (Peng Shaobing et al., 1993). Also Khoddamzadeh and Dunn (2016) found that SPAD readings did not correlate well with N_M in two greenhouse grown chrysanthemum cultivars and differences in N fertilizer rates were difficult to discern and showed both a temporal and cultivar dependency. To our knowledge the Dualex sensor has never been used to predict foliar N in *Chrysanthemum* spp. Chl measured with Dualex behaves similarly as Chl measured with SPAD, but EPhen correlated very good with N_M (Fig. 7). As less photoprotection is needed at the abaxial leaf side, abaxial EPhen was lower in content but correlated mostly better with N_M compared with adaxial EPhen. Total EPhen, however, showed, especially for 'Orlando', a steeper slope and thus a higher distinctive power. For 'Orlando' the correlation is valid for both years, but for 'Maya' small differences occur between 2017 and 2018, despite a high R^2 for when both years are combined.

Contrary to previous research findings on other plant species, NBI had no higher discriminatory power to detect differences in N fertilization levels compared to EPhen alone (Demotes-Mainard et al., 2008). Correlation analysis revealed that mass-based N_M correlated better with mass-based conversions of non-destructive chlorophyll measurements (by dividing these parameters by LMA) (Table 4). This is consistent with other researchers' findings that LMA should be considered when using SPAD to predict the foliar N% (Demotes-Mainard et al., 2008; Meyer et al., 2006). Similar findings account for Chl measured with Dualex. This is not the case for EPhen, which makes this a robust estimator for N_M irrespective of LMA change during the growing season.

NDVI of both 'Maya' and 'Orlando' was higher in 2017 and 2018 compared to 2016 although plant dimensions and nitrogen content were lower. This contrast can be explained by the slightly different scanning procedure: because gaps caused by plant harvest were scanned along with the plants rows in 2016, mean NDVI was lower. This also explains the difference in correlation between NDVI and FW (Fig. 9). Regression lines of 2017 and 2018 of both cultivars are much more similar while the regression lines in 2016 are more distinct. For 'Orlando' there were still slightly different regression lines in 2017 and 2018, indicating other parameters/stress factors must have influenced NDVI, for instance soil brightness due to different soil water content (Huete et al., 1985). NDVI correlated weakly ($r < 0.5$) and/or negatively with plant N% (Table 5), which can be elucidated by the contrasting evolution of biomass and plant N% during the growing season (Figs. 2 and 5A–F). Since

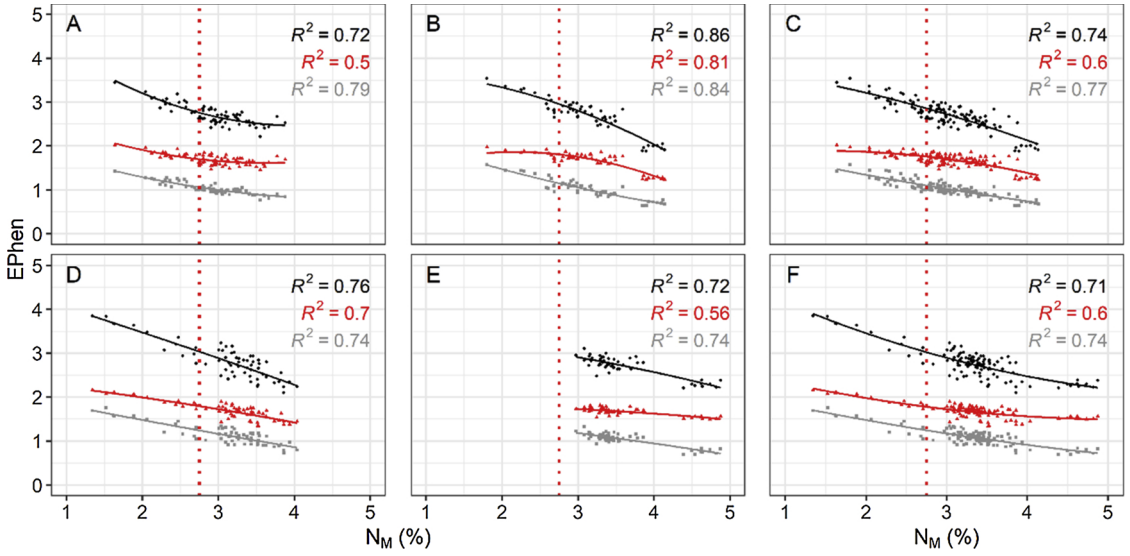

Fig. 7. Relationship between the mass-based N content (N_M) and total (black dots), adaxial (red triangles) and abaxial (light grey squares) epidermal polyphenolics measured with Dualex (EPhen). (A: 'Maya' 2017, B: 'Maya' 2018, C: 'Maya' overall, D: 'Orlando' 2017, E: 'Orlando' 2018, 'F': 'Orlando' overall). The dotted vertical line at 2.75% N_M represents the lower sufficiency limit for *Chrysanthemum*. Each point corresponds to one replicate of 10 pooled leaves in 2016 and 2017 and 15 in 2018.

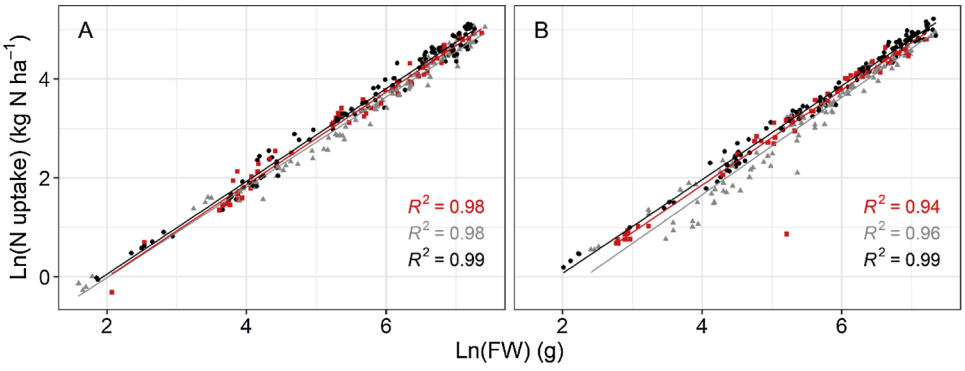

Fig. 8. Linear regression of ln transformed N uptake vs. ln transformed FW for (A) 'Maya' and (B) 'Orlando' in 2016 (black dots) and 2017 (grey triangles). Regression equations for 2016, 2017 and 2018 respectively for 'Maya' are ln(N uptake) = -2.08 + 0.98 ln(FW), ln(N uptake) = -2.31 + 0.98 ln(FW) and ln(N uptake) = -1.84 + 0.93 ln(FW) and for 'Orlando' ln(N uptake) = -2.17 + 1.00 ln(FW), ln(N uptake) = -2.96 + 1.10 ln(FW) and ln(N uptake) = -1.98 + 0.96 ln(FW).

Table 5
Pearson correlation coefficient (r) of NDVI vs. destructively measured plant parameters (FW, DW, plant N) and other quality parameters (height and diameter) in *Chrysanthemum* cvs. 'Maya' and 'Orlando' for 2016–2018 (n° of data points between brackets).

	Maya 2016	Maya 2017	Maya 2018	Orlando 2016	Orlando 2017	Orlando 2018	Overall
NDVI vs. FW	0.93 (72)***	0.80 (66)***	0.89 (45)***	0.93 (70)***	0.76 (72)***	0.92 (45)***	0.74 (370)***
NDVI vs. DW	0.90 (72)***	0.80 (66)***	0.88 (45)***	0.94 (71)***	0.75 (72)***	0.92 (45)***	0.74 (371)***
NDVI vs. plant N (%)	−0.44 (72)***	−0.50 (66)***	−0.82 (45)***	−0.39 (72)***	−0.02 (72)ns	−0.8 (45)***	−0.51 (372)***
NDVI vs. height	0.94 (63)***	0.90 (66)***	0.91 (45)***	0.87 (63)***	0.87 (72)***	0.91 (45)***	0.77 (354)***
NDVI vs. diameter	0.94 (63)***	0.90 (66)***	0.92 (45)***	0.91 (63)***	0.87 (72)***	0.96 (45)***	0.84 (354)***
NDVI vs. N uptake (kg N ha^{-1})	0.89 (72)***	0.78 (66)***	0.85 (45)***	0.92 (71)***	0.73 (72)***	0.90 (45)***	0.68 (371)***

ns = non-significant, *= significant at P ≤ 0.05, **= significant at P ≤ 0.01.
*** = significant at P ≤ 0.001.

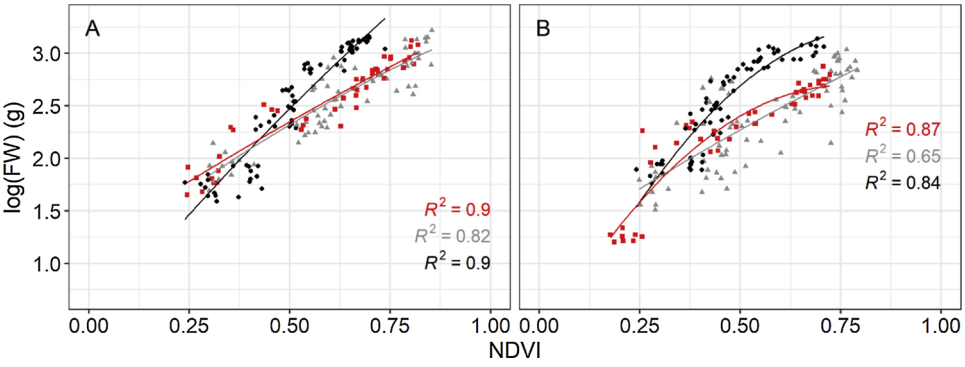

Fig. 9. Correlation between fresh weight (FW) and NDVI measured with GreenSeeker in 2016 (black), 2017 (grey) and 2018 (red) for 'Maya' (A) and 'Orlando' (B). Each symbol corresponds to the DW of one replicate of 2 sampled plants and the NDVI of the total plot. Regression equations for 'Maya' are log (FW) = 0.55 + 3.82 NDVI, 1.23 + 2.15 NDVI and log(FW) = 1.26 + 2.13 NDVI and for 'Orlando' log(FW) = −0.118 + 8.01 NDVI – 4.78 NDVI^2, log(FW) = 1.21 + 2.10 NDVI and log(FW) = 0.24 + 6.44 NDVI - 4.23 NDVI^2 for 2016, 2017 and 2018 respectively.

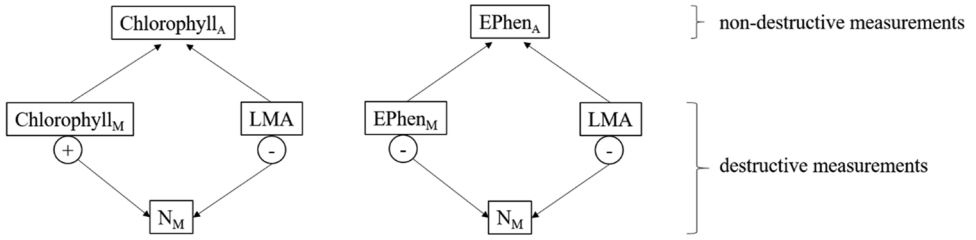

Fig. 10. Influential parameters of non-destructively measured parameters chlorophyll$_A$ and EPhen$_A$ (area-based) measured with SPAD and Dualex.

Chrysanthemums change from herbaceous to more sclerified stems during their development, it is self-evident that plant N% will reduce over time. Khoddamzadeh and Dunn (2016) found that NDVI determined by a handheld GreenSeeker correlated with foliar N of the two researched *Chrysanthemum* cvs. for selected time points. These results suggest that optical sensing might have potential for chrysanthemum although no general seasonal approach was given.

4.3. Applicability of chlorophyll meter readings, Greenseeker for assessing plant N status

Chl and SPAD were positively and tightly correlated (R^2 = 0.86 and R^2 = 0.79 for 'Maya' and 'Orlando' respectively, P < 0.001), thus both leaf-clip meters can be used reciprocally for measuring the chlorophyll content in *Chrysanthemum* leaves. However, a big disadvantage of non-destructively measuring chlorophyll is the urge for an LMA-correction to improve the correlation with foliar N. This LMA-correction is necessary because LMA and mass-based chlorophyll content, both influencing parameters of the non-destructively measured chlorophyll content, are respectively negatively and positively correlated with N_M. This is not the case with EPhen, where both influential parameters (EPhen$_M$ and LMA) are negatively correlated with N_M (Fig. 10). EPhen values at the early start of the growing season in 2018 did also not negatively influence the correlation with N_M, contrary to Chl (Fig. 6C). This makes

EPhen a reliable parameter for year-round prediction of foliar N content.

As strong (negative) correlations were found between foliar N_M content and abaxial EPhen for both cultivars, we can consider this type of measurements as most valuable to non-destructively predict leaf N%. The advantage of this high one-sided correlation is that it is less time consuming and that measurements of both leaf sides do not have to be summed afterwards, which improves in-field usability. Dualex can thus be used as a quick and easy tool to identify an N deficit before plant quality and dimension is affected. When using the lower sufficiency limit of foliar N_M of 2.75% in *Chrysanthemum,* $EPhen_{ab}$ values below 1.09 for 'Maya' and 1.38 for 'Orlando' indicate an N deficit, however, based on our research the sufficiency limit is cultivar and sometimes growing season specific. Since *Chrysanthemum* growers often grow multiple cultivars, obtaining cultivar-specific N sufficiency values is expensive, time-consuming and therefore unfavorable. The use of on-site well fertilized reference plots can be of help to improve the accuracy between actual crop N and raw sensor readings (Goffart et al., 2008). Hence, also other influencing parameters, e.g. other nutrient deficits or drought stress, can be eliminated.

Although statistical analysis indicated that regression equations were slightly different over the years, overall correlations between N uptake and FW, DW, height and diameter were very good, implying that these parameters can be used to predict the amount of additional N needed to attain a certain diameter at the end of growing season. The amount of available mineral nitrogen in the soil and the expected natural mineralization should then be subtracted to determine a suitable additional side dressing. Since N-uptake was less correlated with NDVI compared to other non-destructive and easy to measure parameters, height and diameter, we do not consider GreenSeeker as a must-have for the improvement of N fertilization in *Chrysanthemum*. Ji et al. (2017) found that implementing cumulative growing degree days improved the relation between NDVI and biomass over two growing seasons, however, this cancels out the advantages of quick measuring tools. Nevertheless it can be more convenient to scan an entire field with a - whether or not tractor-mounted - GreenSeeker instead of measuring individual plant diameters to look for within-field quality and fertilizer need variance. For convenient N status assessment on field level a Multiplex Research device (FORCE-A, Orsay, France), which is a portable plant sensor measuring polyphenols, can be of help, but applicability for *Chrysanthemums* should be researched.

5. Conclusion

In conclusion, we have shown that non-destructive optical sensors can be valuable decision-support methods to assess the N status of *Chrysanthemum* cvs. 'Maya' and 'Orlando'. At leaf level, we have shown that LMA affects the correlation of N_M and Chl, which is not the case with EPhen. Hence, (abaxial) EPhen measured with Dualex can function as a reliable proxy for foliar N content. At canopy level, NDVI can be useful to identify the need for additional side dressing to achieve the desired plant quality, but is not informative as a proxy for plant N concentration. Non-destructive optical sensors can thus support *Chrysanthemum* growers to achieve a qualitative end product, avoiding overfertilization and associated nitrate leaching.

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Appendix A

Fig. A1. Open–field *Chrysanthemums* production: pots are partly buried. After 2–4 weeks the roots reach the surrounding soil and start to absorb water and nutrients.

Table A1
Dates of potting, transplantation and fertilization for 'Maya' and 'Orlando' in 2016–2018.

	2016		2017		2018	
	'Maya'	'Orlando'	'Maya'	'Orlando'	'Maya'	'Orlando'
potting date + placement on container field	May 18	May 18	May 18	June 1	May 14	June 6
transplantation to the field	June 15	June 15	June 15	June 26	June 14	June 25
1 st pot dressing	–	–	June 1	June 21	June 4	June 19
2nd pot dressing	–	–	June 12	June 28	–	–
3rd pot dressing	–	–	June 20	July 5	–	–
4rd pot dressing	–	–	June 28	July 12	–	–
5th pot dressing	–	–	July 5	July 18	–	–
1 st soil dressing	June 22	June 22	July 12	July 12	July 20	July 20
2nd soil dressing	July 25	July 25	July 18	July 18	August 10	August 10
3rd soil dressing	August 29	August 29	July 27	July 27	September 3	September 3
4rd soil dressing	–	–	August 11	August 11	–	–

Appendix B

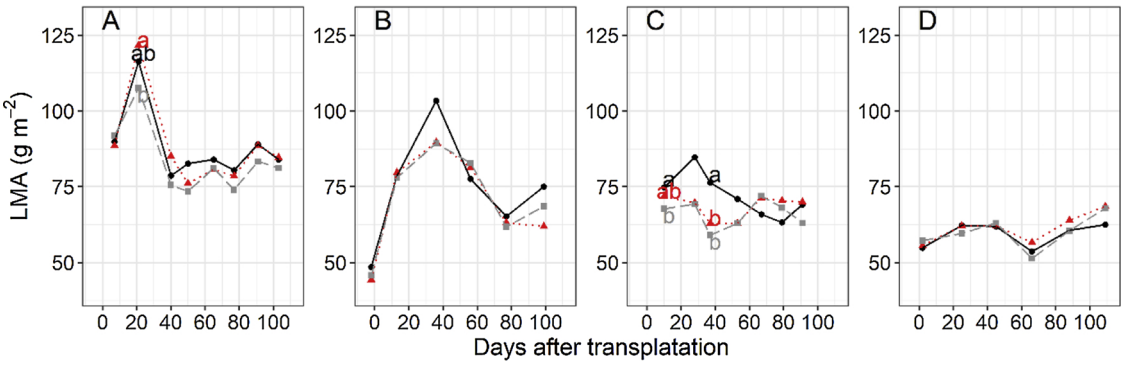

Fig. B1. Changes in LMA of young full grown leaves of 'Maya' (A: 2017, B: 2018) and 'Orlando' (C: 2017, D: 2018). Full black, dotted red and dashed grey lines represent N0, N1 and N2 treatments respectively. Each point is the mean of 3 replicates of 10 (2017) or 15 (2018) pooled leaves. Means labelled by the same letter did not significantly differ at $P < 0.05$. If no letters are present, no significant differences were present at that time point.

Table B1
Pearson correlation coefficients r of N uptake vs. destructively measured plant parameters (FW, DW) and other quality parameters (height, diameter) in two *Chrysanthemum* cvs. 'Maya' and 'Orlando' for 2016–2018 (n° of data point between brackets).

	Maya 2016	Maya 2017	Maya 2018	Orlando 2016	Orlando 2017	Orlando 2018	Overall
N uptake vs. FW	0.96 (72)***	0.98 (66)***	0.98 (54)***	0.99 (79)***	0.99 (72)***	0.98 (45)***	0.97 (397)***
N uptake vs. DW	0.95 (72)***	0.97 (66)***	0.98 (54)***	0.98 (80)***	0.98 (72)***	0.98 (54)***	0.97 (398)***
N uptake vs. height	0.94 (72)***	0.93 (66)***	0.95 (54)***	0.92 (79)***	0.88 (72)***	0.9 (54)***	0.92 (397)***
N uptake vs. diameter	0.95 (72)***	0.97 (66)***	0.95 (54)***	0.94 (79)***	0.93 (72)***	0.96 (54)***	0.91 (397)***

ns = non-significant, * = significant at $P \leq 0.05$, ** = significant at $P \leq 0.01$.
*** = significant at $P \leq 0.001$.

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