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Growth tracking of basil by proximal remote sensing of chlorophyll fluorescence in growth chamber and greenhouse environments

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ABSTRACT

Remote sensing is a promising tool for plant phenotyping and precision farming, as it allows for non-invasive, fast and automated measurements of relevant plant traits with spatial and temporal resolution. The simplest and most used remote sensing application in the field is to use reflectance vegetation indices, based on the optical properties of chlorophyll, as indicators of variables of interest. However, the applicability is limited by their sensitivity to environmental conditions and canopy structure. Another remotely sensed signal related to chlorophyll is chlorophyll fluorescence. Compared to reflectance it is plant specific and directly linked to plant physiological processes; but it is also weak, which complicates its use for in-field applications. This study evaluates the performance of an active proximal remote sensing system utilizing the chlorophyll fluorescence ratio method, measuring the ratio of red fluorescence to far-red fluorescence (termed SFR), for the assessment of growth and biomass as an alternative or complement to reflectance vegetation indices.

Basil plants were subject to chlorophyll fluorescence and weight measurements periodically throughout commercial growth cycles, both in a laboratory and commercial greenhouse environment. In the laboratory, SFR showed a strong linear relationship with dry weight on logarithmic scales. Further characterization of the method indicated that it is independent of background light and the same growth dynamics is obtained irrespective of point in time during chlorophyll fluorescence induction. The same trend that was observed in the laboratory was also observed in the greenhouse, but varying background light from the sun and from supplemental lighting added complexity that needs to be addressed in further studies. To our knowledge, the strong link between SFR and biomass, both in a closed environment and greenhouse setting, has not so clearly been demonstrated on canopy level before. Owing to the simplicity of the method, being relatively cheap and fast, it has potential for commercial applications.

1. Introduction

More efficient methods for plant phenotyping and precision agriculture are needed to meet future requirements in crop production and environmental sustainability. See Kumar et al. (2015), Fiorani and Schurr (2013), Deery et al. (2014), and Li et al. (2014) for recent introductory reviews on plant phenotyping and the concept of precision agriculture is explained by e.g. Diacono et al. (2013). Methods using optical remote sensing technology are particularly promising as they allow for non-invasive, fast and automated measurements with both spatial and temporal resolution in the field. They are based on transmittance, reflectance or fluorescence signals from the plants, which contain information about agronomic and physiological traits.

Canopy reflectance is the most accessible and widely studied signal for field applications. It is a function of leaf morphological properties, absorption of light by pigments, and plant architecture, such as varying arrangements of leaves, branches and stems (Winterhalter et al., 2013). The optical properties of chlorophyll, with strong absorption in the blue and red wavebands, are clearly observed in the reflectance spectrum. Since chlorophyll is involved in photosynthesis and other physiological processes, the pigment provides a link between remote sensing observations and plant physiology (Schlemmera et al., 2013). For example, chlorophyll is closely related to rubisco, which is the main sink for nitrogen, and thereby chlorophyll content is a good indicator of nitrogen status (Chappelle et al., 1984). The most common approach to estimate crop parameters from reflectance spectra is by the use of empirically derived reflectance vegetation indices (rVIs). These are simple formulas that consist of a combination of two or more

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reflectance wavebands. Typically two wavebands, one that is correlated and one that is uncorrelated to the parameter of interest, are expressed as a ratio or normalized difference in order to minimize the variability induced by external factors.

rVIs based on chlorophyll have been derived to assess nitrogen (N) status, green biomass, photosynthetic capacity, leaf-area index (LAI), plant stress and other important crop parameters. Many studies have demonstrated the utility of these indices and, as a result, commercial sensor systems for routine monitoring in the field have been developed, such as the Yara N Sensor™ ALS (Yara International ASA, Dülmen, Germany), the GreenSeeker™ RT200 (Trimble, Sunnyvale, USA), and the Crop-Circle ACS-470 Active Crop Canopy Sensor (Holland Scientific, Lincoln, USA). However, the full potential is yet to be explored. On the canopy scale, reflectance indices are affected by both sampling conditions and chlorophyll independent canopy features. Soil interference, illumination conditions (e.g. solar illumination angle and shadowing), sensor position (e.g. sensor view angle) and plant structure (e.g. leaf inclination angle, species-specific canopy architecture and leaf surface features) are some of the factors that influence the reflectance spectrum (Royo and Villegas, 2011)). They have mostly been neglected, leading to substantial imprecisions in the estimates and to the need of site-specific calibration (Jones, 2014). This has clearly limited the use of rVIs in plant phenotyping and the technical challenges need to be addressed. Active sensors, that are not dependent on the sun but possess their own light-emitting units, are less sensitive to varying illumination conditions and thereby improve robustness and timeliness of operation. The commercial instruments listed above are all based on active sensing technology. Major improvements can also be made by correcting for canopy structure and illumination conditions using canopy reflectance models based on radiative transfer theory (Feret et al., 2011; Knyazikhin et al., 2012), but more research is required.

An important limitation of rVIs based on chlorophyll is reflectance saturation. At high crop densities the reflectance reaches an asymptote making the rVI insensitive to changes in chlorophyll content. By employing spectral bands outside the major absorption bands, it is possible to retain sensitivity longer. For example, indices based on the red-edge- instead of the red region have shown better performance in this aspect (Nguy-Robertson et al., 2012). The performance can also be improved by using more than just a few wavebands. Various techniques have been tested for the selection of critical wavebands, including 2D correlation plots (Darvishzadeh et al., 2008), partial least square regression (Serbin et al., 2012) and principal component analysis (Dreccer et al., 2014).

The chlorophyll content of plant canopies can also be assessed remotely by measuring chlorophyll fluorescence (ChlF). ChlF is the red- and far-red emission by Chl *a* upon absorption of light. It is a de-excitation pathway, competing with photochemical conversion and heat dissipation, in the photosynthetic apparatus. The ratio between red and far-red fluorescence (RF/FRF), termed SFR for Simple Fluorescence Ratio in this study and elsewhere (Tremblay et al., 2012), is sensitive to chlorophyll content because red- but not far-red fluorescence is readily reabsorbed. As such, the ratio decreases with increasing chlorophyll content (Buschmann, 2007; Gitelson et al., 1998; Lichtenthaler and Rinderle, 1988).

The SFR parameter is offered by fluorometers using the so-called laser-induced two-wavelength ChlF technique (Tremblay et al., 2012). Most of them are hand-held devices, measuring single leaves at close distances, which cannot be used on-the-go. One exception is the tractor-mounted Planto N-Sensor (Planto GmbH, Leipzig, Germany), which can handle a measurement distance of 3–4 m between the sensor and the canopy. It measures ChlF, induced by red (630 nm) laser light, in the red (690 nm) and far-red (730 nm) wavebands. The instrument has been evaluated in a number of field studies: the ratio of the two wavebands showed strong correlation with aboveground N content of oilseed rape, with an accuracy comparable to reflectance-based methods (Thoren and Schmidhalter, 2009); and a strong relationship was also observed between SFR and both total aerial N and aerial dry mass in wheat, but the coefficient of determination was slightly lower when compared to some reflectance indices (Mistele and Schmidhalter, 2010). Other than that, there are only a few studies looking on SFR on canopy level using active sensing (Ač et al., 2015). Most of them investigate how it correlates with stress factors such as drought (Dahn et al., 1992; Valentini et al., 1994), nitrogen deficiency (Kuckenberg et al., 2009), pathogen infections (Kuckenberg et al., 2009), and temperature (Thoren et al., 2010).

A rather new commercially available fluorometer that potentially can be used for screening large number of plants in the field is MULTIPLEX RESEARCH™ (Force-A, Orsay, France; Tremblay et al., 2012). In addition to chlorophyll indices, it measures indices related to polyphenol content using the ChlF screening method. The method compares ChlF induced by two different excitation lights, one reference light that is not screened and one sampling light that is screened by the polyphenol of interest. For example, a smaller fraction of UV light compared to red light reaches the chlorophyll in the mesophyll because it is absorbed by flavonols in the epidermis. LEDs of four different colors are used as excitation lights: UV (375 nm), blue (450 nm), green (530 nm) and red (630 nm). Flavonol and chlorophyll have opposite N-dependence, suggesting that a combined index is a better indicator of N status than chlorophyll alone. The combined index (NBI, nitrogen balance index; Cartelat et al., 2005) proved to be better than the chlorophyll index in discriminating N levels applied to turfgrasses, and it was superior to reflectance-based indices (Agati et al., 2015; Agati et al., 2013). Padilla et al. (2016) demonstrated the applicability on vegetable crops by showing that the fluorescence indices for chlorphyll (SFR), flavonols (FLAV) and nitrogen (NBI) are able to distinguish deficient from optimal crop N status in cucumber. The Multiplex sensor was developed as a hand-held device measuring a small surface (8 cm in diameter) at 10 cm distance from the light source. A tractor-mounted version, which is able to work at 20–30 cm distance, has more recently been developed for on-the-go applications. It has been shown to be capable of assessing chlorophyll, nitrogen and anthocyanin levels on grapevines and grape berries in the field (Diago et al., 2016; Bramley et al., 2011).

ChlF has advantages over reflectance for remote sensing. The signal is plant specific and therefore less sensitive to soil interference. It is also more directly linked to plant physiological processes. However, the signal is weak and noisy, owing to the small quantum yield of ChlF (typically 0.5–3% and not exceeding 10%; Brody and Rabinowitch, 1957; Latimer et al., 1956; Krause and Weis, 1991), making remote measurements from mobile platforms technically challenging. Similar to reflectance-based methods the signal is also sensitive to saturation at high crop densities (Gitelson et al., 1998).

There are alternative approaches to the ChlF ratio method and the ChlF screening method. Measuring variable ChlF by using PAM (pulse amplitude modulation) technology is widely used in plant science for the assessment of photosynthetic functioning and crop health status (Tremblay et al., 2012). It involves measurements under light saturating conditions, it is sensitive to measurement distance and dark-adaption is required for full analysis. These properties are difficult to accommodate for in the field. A related technique called laser-induced fluorescence transients (LIFT) have been developed to remotely measure photosynthetic properties at a distance of up to 50 m (Kolber et al., 2005), and it was recently tested for the first time in an agriculture setting (Raesch et al., 2014). Passive remote sensing of chlorophyll fluorescence is possible through the infilling of radiation in the Fraunhofer lines in the

solar spectrum. This approach, called SIF (sun-induced fluorescence), has received a lot of attention in the last couple of years as it can be used for satellite-based monitoring (Porcar-Castell et al., 2014). The primary goal is to use the SIF signal as a proxy of photosynthesis and link it to gross primary productivity. Although promising, the new SIF methodology introduces many scientific questions that remain to be answered. The main questions are related to temporal and spatial up-scaling and what information that can be derived from it (Porcar-Castell et al., 2014).

So far, ChlF has not been widely used for field phenotyping (Tremblay et al., 2012). Most sensor systems are limited to close-distance measurements of single leaves, making the screening process too laborious and time consuming. There is a clear need for canopy-level measurement methods that are fast and cost-effective and that provide valuable information (Deery et al., 2014), which is the focus of this study. The ChlF ratio method studied here, and the ChlF screening method show promise for on-the-go sensor applications. They can bring new information complementary to reflectance remote sensing, which has not provided complete satisfaction alone. Indeed, several studies have shown the potential of combining these two approaches for in-field detection of e.g. stress and N status, as discussed by Tremblay et al. (2012), but more research is needed. This study evaluates the performance of an active proximal remote sensing system measuring SFR for the assessment of growth and biomass. The objectives are: (1) to characterize the fluorescence signal on canopy scale throughout a growth cycle, in particular how it relates to plant growth and biomass and (2) to test the applicability of the sensor system for tracking plant growth in both a laboratory environment and a commercial greenhouse environment, comparing with traditional reflectance-based indices.

2. Materials and methods

2.1. Active proximal remote sensing system

The measuring system consisted of two major components: light source and light sensor. A commercially available horticulture LED lamp (L4S10-series, Heliospectra, Sweden) served as light source. This lamp has seven LED groups with wavelength peaks at 400, 420, 450, 530, 630, 660, 735 nm. The intensity of each LED group can be controlled individually from a computer, making the lamp very flexible in terms of light intensity and spectral composition.

Incident light as well as fluorescence and reflectance from the canopy were measured using high-sensitivity spectrometers (Maya2000 Pro, Ocean Optics, Dunedin, Florida, USA) with an optical resolution of about 1.85 nm (FWHM) in the spectral range of 200–1100 nm. The light was collected via optical fibers (600 μm diameter) whose sensor heads were fitted with optical restrictors (Gershun tube or cosine diffuser) to control the field-of-view (FOV). The sensor(s) measuring canopy fluorescence and reflectance was placed at the lamp level facing downwards (used in growth chamber and greenhouse experiments), whereas the sensor measuring incident light was mounted at the canopy level facing upwards (used in greenhouse experiment).

2.2. Growth chamber experiment

The sensor system was first tested in a controlled environment with no sunlight. The purpose was to characterize the fluorescence signal at the canopy level, how it changes during the growth cycle and how it relates to biomass, without having to cope with varying ambient light. The experiment was conducted during fall 2013 in a controlled environment growth room at Heliospectra Plant Lab (Gothenburg, Sweden) on basil (*Ocimum basilicum*, 'Aroma 2') grown under LED light (16 h photoperiod 8:00–24:00; PPFD approximately 180 $\mu mol\ m^{-2}\ s^{-1}$). Temperatures were 20–26 °C during day and 15 °C during night, and the relative humidity was about 60%. Two batches of plants were grown for growth cycle 1 and 2, respectively (see next paragraph). The only major difference between the batches was the plant density. The first batch started off with 15 pots per tray on 4 trays, whereas the second started off with 19 pots per tray on 6 trays.

Measurements were performed at regular intervals, every one to three days, throughout two commercial growth cycles. During the first growth cycle (GC1) 9 measurements were conducted between 19 and 41 days after seeding (DAS). GC2 contained 15 measurements between 16 and 47 DAS. The time on the day varied from 9:00 to 12:00, with the exception of GC1 DAS 39 and GC2 DAS 45 taking place at 16:00. At each occasion, two full trays of basil were placed in the measurement unit for obtaining the ChlF spectrum. The pots were arranged with the aim to get a closed canopy as seen by the ChlF sensor. The number of pots per tray decreased from about 20 to about 10 as the plants grew bigger. The measurement unit consisted of a Styrofoam box (WDH 70 × 70 × 90 cm) with two LED lamps placed on top and two spectrometers measuring canopy fluorescence mounted at the lamp level facing downwards (Fig. 1). Also at each occasion, three plants were destructed for weight measurements. Dry weights were taken after having the plant material in the oven at 70 °C for three days or more.

ChlF was induced by turning on the LED excitation light, and spectra were recorded continuously for approximately 5 min. By that time the signal had reached the apparent steady-state level following the ChlF induction phenomenon (CFI). Different configurations were tested to characterize the signal (see Table 1): blue light (LED420) and green light (LED530) were used as excitation light, one of the sensors were fitted with a Gershun tube set to 20° FOV and the other with a cosine diffuser having 180° FOV, and measurements were performed both in the absence and in presence of background light.

The intensity of the excitation light at the canopy level was approximately 120 and 190 $\mu mol\ m^{-2}\ s^{-1}$ for LED420 and LED530, respectively, measured without plants in the unit. When background light was on, the excitation light intensity was a bit lower (approximately 83 and 110 $\mu mol\ m^{-2}\ s^{-1}$). LED420, LED450, LED530 and LED630 provided background light with a total intensity of approximately 240 $\mu mol\ m^{-2}\ s^{-1}$.

2.3. Greenhouse experiment

The potential of using this sensing approach and sensor system was further explored in a greenhouse setting, with varying background light from the sun and from HPS lamps added as complicating factors. The experimental site was a commercial greenhouse in the south of Sweden (Lat. 56°N). Two rounds of experiments were conducted, the first in February and the other in May 2014. In February, HPS lamps were turned on and sunlight was weak, which was opposite to the situation in May. Again, basil was used as crop model.

In this greenhouse, plants coming from the nursery are entering the production line in one end. At the other end the plants are harvested. Most growth stages are, therefore, present on the production line at all times, which makes it possible to measure canopy ChlF throughout a growth cycle at once. The experiment took a couple of hours instead of about three weeks, which is required if measuring a single sample moving from one end of the production line to the other.

A custom-made rig holding one LED lamp and two spectrometers was placed on a trolley that normally transports empty

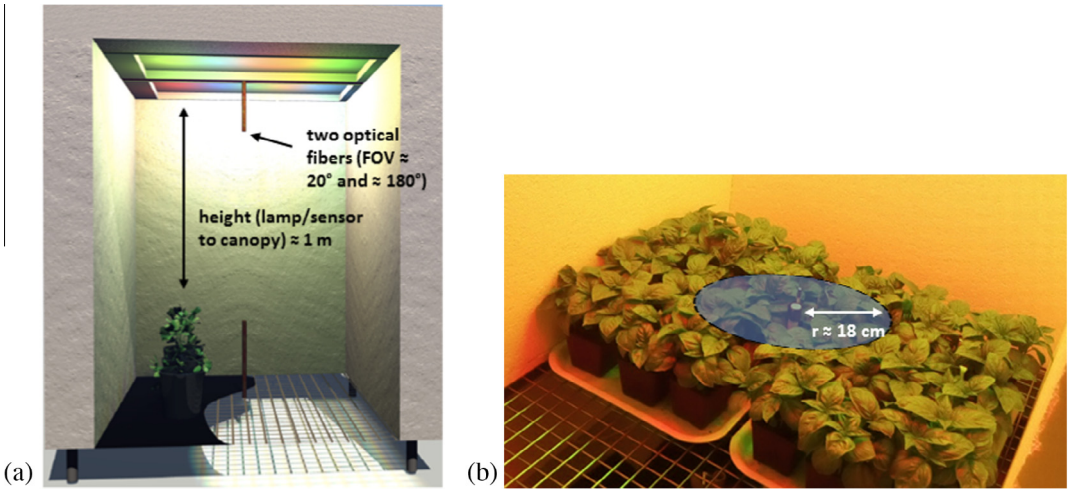

Fig. 1. Set-up in growth chamber experiment: (a) measurement unit equipped with two lamps and two optical fibers for measuring canopy fluorescence and (b) two full trays of basil were placed in the unit for measurements, and the area viewed by the sensor had a radius of approximately 18 cm (the area in the image is for illustration).

Table 1
Sensor system configurations tested in the growth chamber experiment.

Configuration		GC1	GC2
Excitation light	LED420	x	x
	LED530	x	x
Background light	Off	x	x
	On		x
FOV	180	x	x
	20	x	x

troughs back to the beginning of the line after harvest (Fig. 2). The distance from the lamp to the canopy was about one meter. One spectrometer measured ChlF and reflectance from a position close to the lamp with a Gershun tube set to 20° FOV. The other spectrometer measured incident light at the canopy level having a cosine diffuser attached to it.

The measurement procedure was almost the same as in the growth chamber experiment. However, all three blue LEDs (LED400, LED420 and LED450) were used to get a stronger fluorescent signal and for practical reasons fresh weight was obtained, instead of dry weight, in the May experiment. Data was collected by moving the trolley over the production line, stopping every time the seeding date changed for a new measurement. 14 measurements (DAS 15 to 31) were conducted between 14:00 and 20:00 in February and 12 measurements (DAS 16–30) between 15:00 and 18:00 in May (Fig. 8).

The incident intensity of the excitation light was measured to be approximately 66 and 43 μmol m^{-2} s^{-1} in February and May, respectively. The intensity difference was probably due to variations in the set-up, such as the lamp-to-sensor distance. A stronger excitation light was obtained with only one LED group in the growth chamber experiment, primarily because of wall reflectance.

In addition to SFR, several of the most common rVIs were obtained from reflected light spectra and from estimated reflectance spectra (ratio of reflected light to incoming light). NDVI, calculated as $(R_{NIR} - R_{red})/(R_{NIR} + R_{red})$ in the wavebands 665–675 nm (R_{red}) and 740–750 nm (R_{NIR}), from reflected light spectra showed strongest correlation with biomass and is used as a reference method in this paper.

3. Results and discussion

3.1. Growth chamber experiment

Figs. 3 and 4 demonstrate the characteristics of the ChlF data obtained in the measurement unit: Fig. 3 shows the dynamic behavior of CFI, and the reabsorption phenomena is displayed in Fig. 4. Throughout the growth cycle the relative intensity of ChlF shifted towards the far-red peak, and consequently the SFR

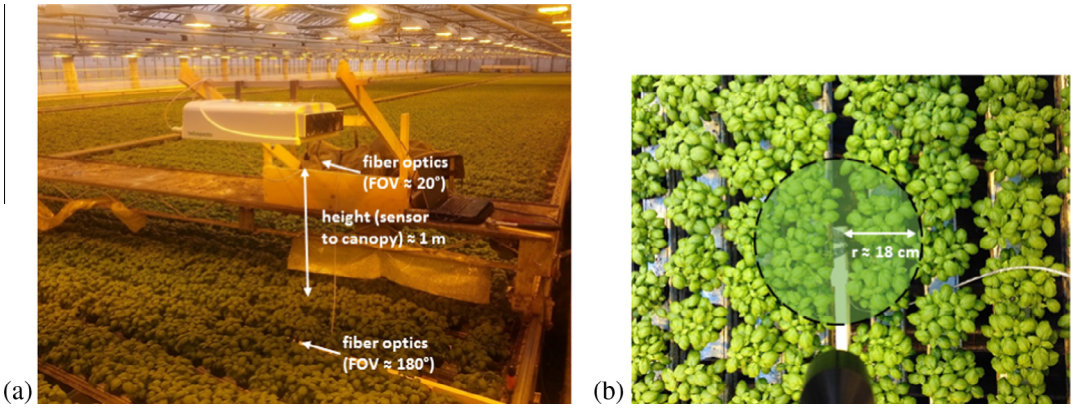

Fig. 2. Set-up in greenhouse experiment: (a) custom-made rig holding one LED lamp and two spectrometer, one for measuring ChlF and one for measuring incident light at canopy level and (b) nadir view of the canopy at one of the measuring spots, including an illustration of the area covered by the FOV of the ChlF sensor.

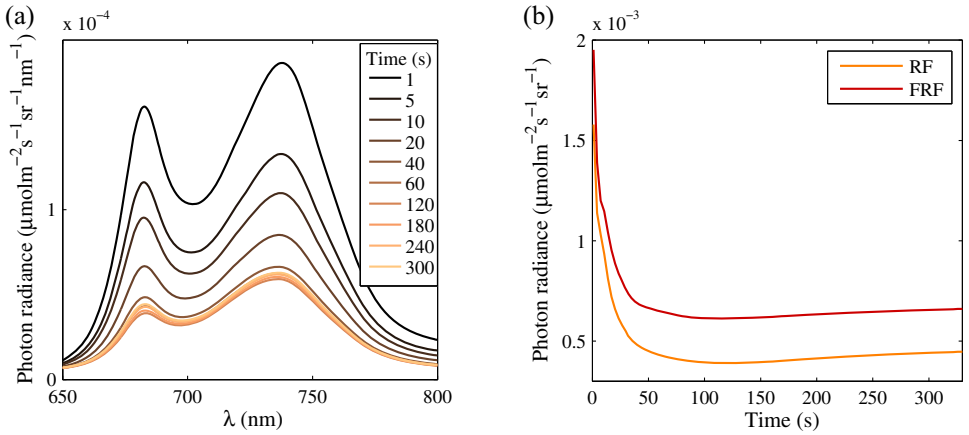

Fig. 3. Spectral readings (a) and RF and FRF (b) during 5 min of LED420 excitation light, following the characteristics of CFI. Data from GC2-LED420 20° FOV DAS30 without background light.

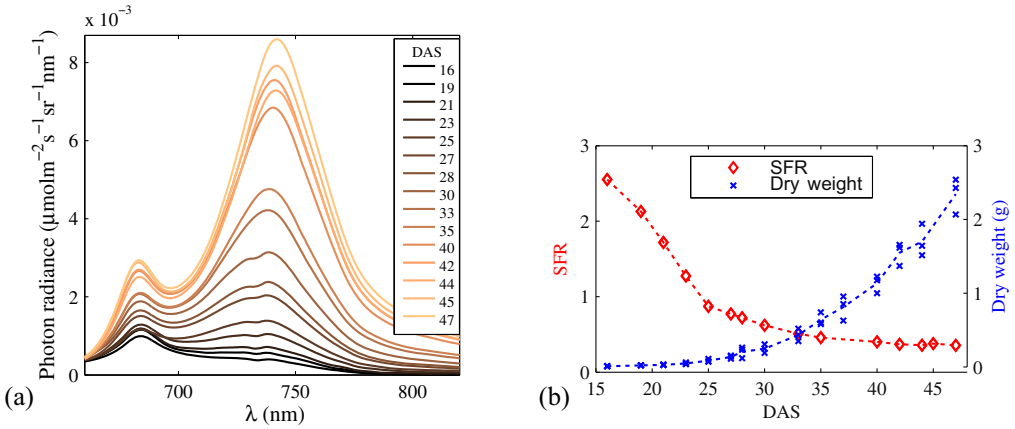

Fig. 4. Steady-state ChlF spectra (a), and SFR and DW (b) throughout a growth cycle. Data from GC2 with 180° FOV.

parameter decreased over time. An inverse curvilinear relationship between SFR and DAS was observed, whereas dry weight increased exponentially (Fig. 4b). By the end of the growth cycle SFR reached saturation, and was no longer sensitive to further changes in DAS or biomass. Saturation occured at around DAS40, which is beyond the harvest day of a commercial growth cycle (typically around 30 days).

The results of the different configurations are compared in Fig. 5. SFR induced by blue light shows a larger dynamic range compared to green light (Fig. 5a–b). This is probably related to different penetration depths. Green light excited ChlF originates on average from deeper layers of the canopy, because of higher transmittance, which yields a smaller SFR as more light is reabsorbed (Buschmann, 2007).

FOV also appears to have an effect on the dynamic range. In GC1-LED530, GC2-LED420 and GC2-LED530, SFR obtained with 180° FOV was larger compared to 20° FOV in the beginning of the growth cycle. By the end of the growth cycle there was no clear difference between the two configurations. Thus, a larger FOV seem to be beneficial for the dynamic range. This was not observed in GC1-LED420 where the readings with different FOVs were similar throughout the whole growth cycle (Fig. 5a). It is reasonable to think that the 20° FOV sensor gives less consistent data in the beginning of the growth cycle as it only sees a fraction of the targeted surface and is therefore sensitive to positioning when the surface is heterogenous. A small difference in how the pots were placed in the measurement unit could have changed the amount of plant material seen by the sensor, which might explain why the third data point in GC2-LED420 and GC2-LED530 20° FOV were larger than the second (Fig. 5b). A larger FOV is more robust in that sense. However, in an environment with varying ambient light, such as a greenhouse, it is probably advantageous to restrict the FOV to ensure that the signal originates from the canopy, which is why the 20° FOV optical restrictor was employed in the greenhouse experiment.

The added background light in GC2 did not clearly influence the SFR value (Fig. 5c), suggesting that it is independent of ambient light. The effect of light intensity on SFR is contentious; some studies report a light dependence (Valentini et al., 1994; Agati et al., 1995), whereas other report no or only a minor influence of ambient light (Gunther et al., 1994). Thoren et al. (2010) adressed the lack of consensus by investigating the issue thoroughly under both field and laboratory conditions. They observed no influence at low light, but above a certain level SFR decreased linearly. The threshold light intensity correlated with saturation of photosynthesis in the upper leaf layers of the canopy. This is not contradictory to our results since the light intensity did not reach saturating levels.

SFR did not change dramatically during the induction kinetics; there was a decreasing trend, which was more pronounced towards the end of the growth cycle. For example, SFR from GC1, both 20° and 180° FOV, decreased 10–30% during CFI (Fig. 6). This is similar to what is expected on single leaves (normally 20–30%),

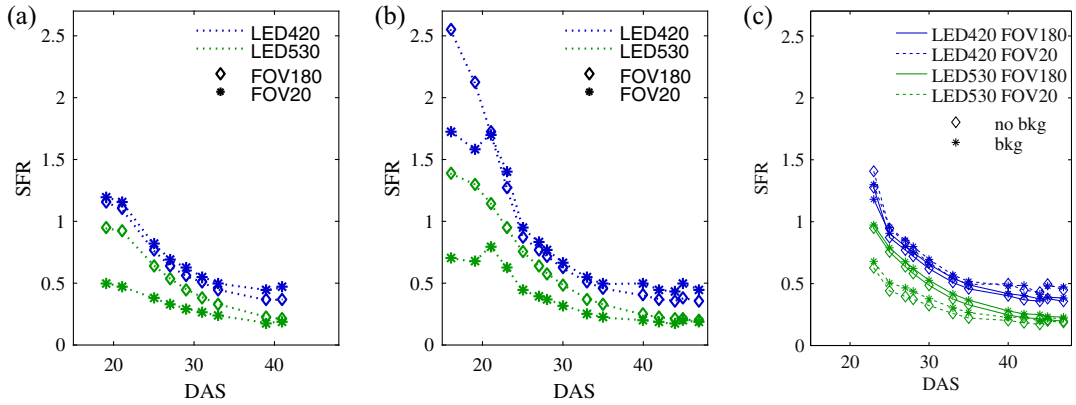

Fig. 5. SFR vs DAS of (a) GC1, (b) GC2 and (c) GC2 with and without background light.

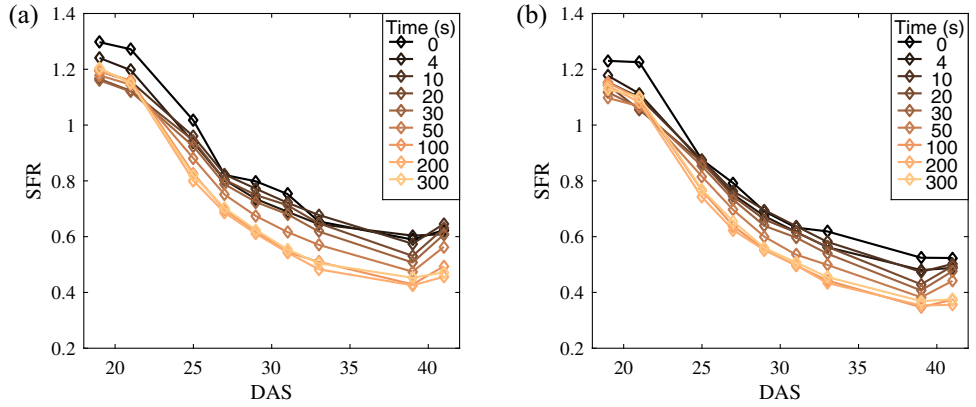

Fig. 6. SFR vs DAS of SFR obtained at different time points of the measurement, i.e. during the induction kinetics. Data from GC1 with 20° FOV (a) and 180° FOV (b).

and the decrease is probably related to a faster rate of induction at the leaf surface compared to the lower part of the tissue (Buschmann, 2007). Importantly, although it changes slightly, SFR seems to have the same dynamic behavior irrespective of point in time during CFI, which means that it is not necessary to wait until apparent steady-state as long as the time point is constant. Similarily, the correlation between chlorphyll content and SFR on single leaves is valid no matter whether the ratio is measured at the maximum level (Fm) or the steady-state level (Fs) (Lichtenthaler and Babani, 2004). This allowed us to take faster measurements in the greenhouse experiment, which was advantageous because of the varying ambient light.

The SFR parameter shows an inverse curvilinear relationship with dry weight using linear scales. When transforming to logarithmic scales, the data fit a linear regression line. Fig. 7 shows log-log plots of dry weight as a function of SFR from both growth cycles including both FOV configurations. Coefficients of determination, root-mean-square errors and normalized root-mean-square errors (normalized by the range of the measured data) are also shown; the correlation is slightly stronger in the 180° FOV data. The results indicate that SFR can be used as a non-destructive indicator of growth and biomass in a growth chamber environment, at least for small to moderate plant densities, which seems be sufficient for crops like basil. Notice, however, that the regression line may change between growth cycles, particularly if the growth conditions are varied. In GC1 the slopes of the regression lines are steeper, potentially attributed to the extra spacing between pots in the growth units which resulted in faster biomass growth. This shows that a calibration method is needed for quantification.

3.2. Greenhouse experiments

Fig. 8 shows ambient incident light, one spectrum from each measurement, in the February and May experiments. The spectra in February have the characteristic features of HPS light. In May, the HPS lamps were turned off and sunlight was stronger, which led to larger variations in PAR, both during and in between the measurements. Variation during a measurement is problematic since the ambient light measured before or after (in this case before) CFI is used for subtracting the influence of background light. Changes in background light level between the reference and sample recordings disturbs the signal and, therefore, the time between measurements needs to be short to decrease that risk. The results in the growth chamber experiment were helpful as they allowed us to reduce and choose the time between induction of ChlF and sample recording.

The fluorescence signal was indeed disturbed by variations in ambient light during the measurements, as can be seen in Fig. 9. The figure shows how the SFR parameter value varies within the first seconds of CFI. Three measurements during the May experiment (DAS 18, 24 and 27) were clearly disturbed more than the others, and were therefore omitted in the regression analysis (Fig. 10b).

The best linear fit between weight (dry weight or fresh weight) and SFR using logarithmic scales was obtained by calculating SFR on data recorded about 7 s after the onset of excitation light in both experiments. The data and the regression lines are shown in Fig. 10a and b. The data is quite noisy, but there is a linear trend. The coefficient of determination is similar in both the February

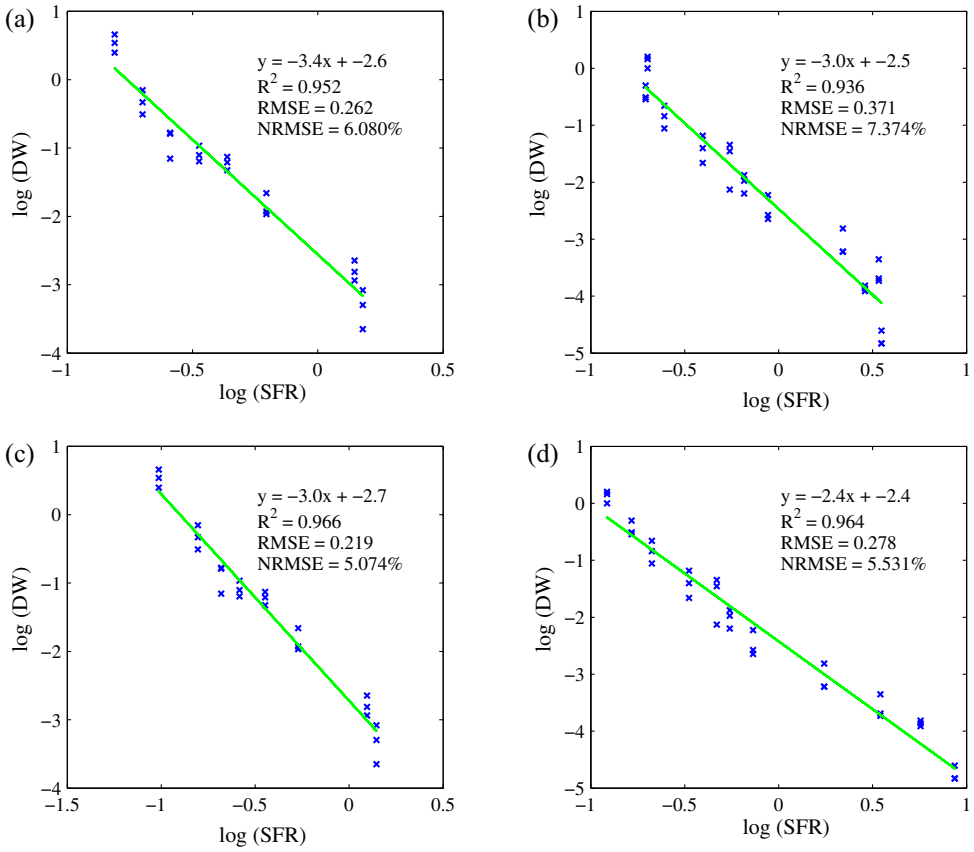

Fig. 7. Log(DW) vs log(SFR) of (a) GC1 20° FOV, (b) GC2 20° FOV, (c) GC1 180° FOV and (d) GC2 180° FOV.

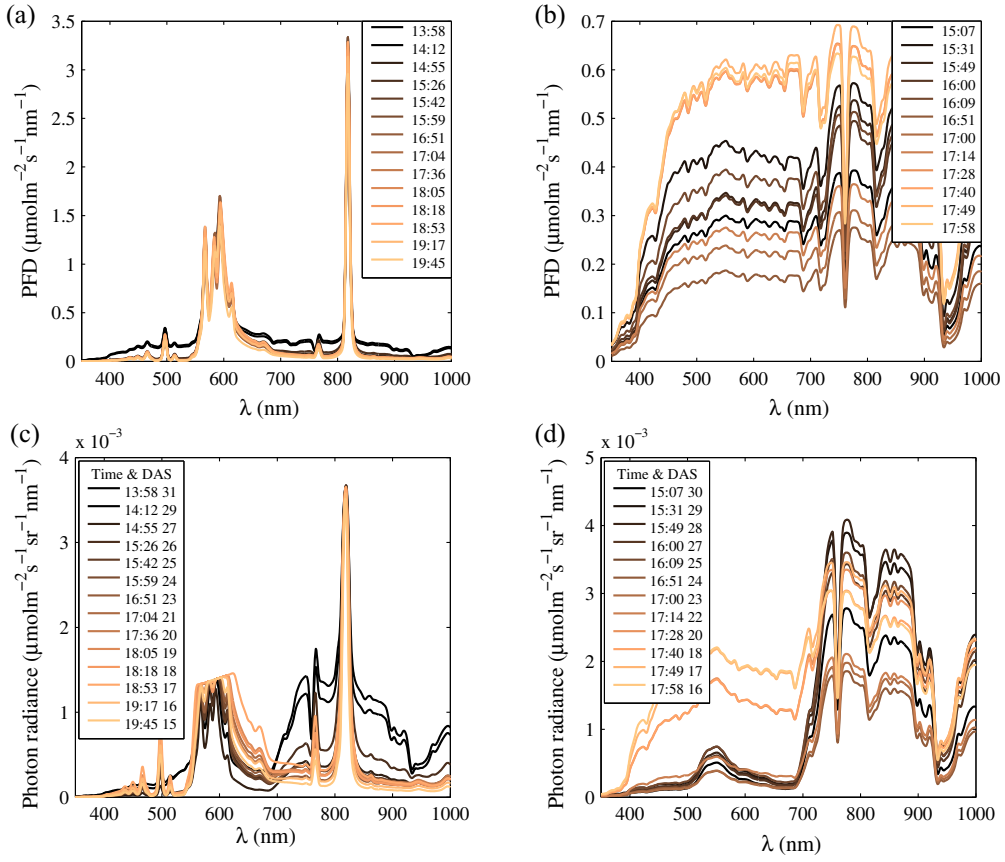

Fig. 8. Incident and reflected light on the canopy in February (a and c) and May (b and d). One spectrum from each measurement. The spectrum is saturated in the green waveband in (c).

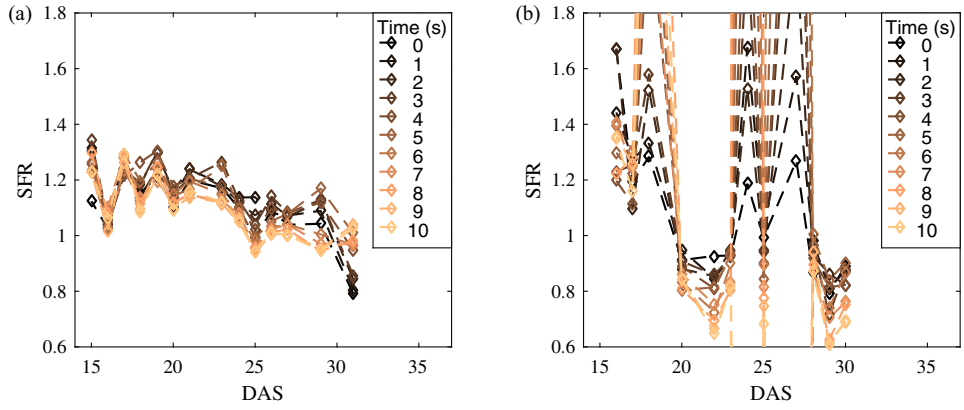

Fig. 9. SFR vs DAS of SFR obtained at different time points of the measurement, i.e. during the induction kinetics. Data from February (a) and May (b) greenhouse experiments.

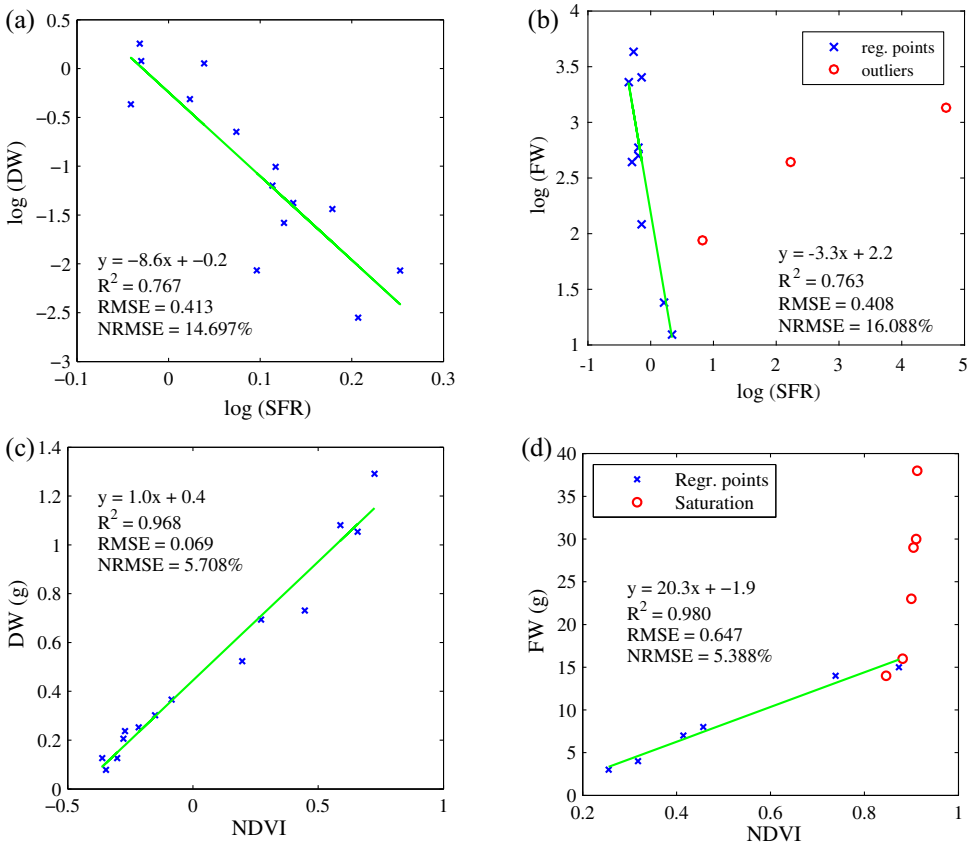

Fig. 10. Weight (DW, log(DW), FW or log(FW)) vs different remote sensing indices – log(SFR) (a, b) and NDVI (c, d) – from the February (a, c) and May (b, d) experiments. A few data points are omitted from the linear regressions because of large changes in ambient light (b: DAS24 and DAS27) and index saturation (d: DAS24-DAS30).

and May data, but three data points in the May data set are omitted due to large disturbances from variations in ambient light, as mentioned. The traditional NDVI parameter, used as reference, outperformed SFR in both February and May, but in May it saturated already at around DAS 23 (Fig. 10c and d). The results indicate that the SFR index is less sensitive to saturation compared to NDVI, supporting the hypothesis that fluorescence can provide additional information compared to traditional rVIs at high crop densities (Rossini et al., 2016).

The robustness to changes in ambient light needs to be improved to enable the method to be employed in such environments. It would likely benefit from a faster measurement procedure. There was an approximately 1.5 s delay between the last reference and first sample recording caused by slow lamp communication. In both experiments the best fits were obtained when the time between reference and sample recordings was small, within a couple of seconds. A shorter delay might have given an improved coefficient of determination, more similar to the growth chamber experiment. Another possibility is to simply limit data collection to times when background light variations are small, and thereby circumvent the problem.

4. Conclusions

Since SFR is sensitive to chlorophyll content we hypothesized that it is sensitive to growth and biomass on a canopy level. This study clearly shows that this is the case in a closed environment for small to moderate plant densities, and that the relationship is

linear on a logarithmic scale. Further, the method seems to be independent of ambient light, at least for non-saturating light levels, and it seems as awaiting steady-state chlorophyll fluorescence is not required. The data from the greenhouse experiment is noisy, due to variations in ambient light during the measurements which disturb the signal, but the trend is similar. Making the measurement procedure faster, particularly the lamp communication, will likely yield a more clear relationship between SFR and dry weight. Interestingly, saturation was not observed in SFR, as opposed to NDVI, in the May greenhouse experiment.

Further studies are needed to improve the method for handling varying ambient light. Calibration practices, e.g. whether site-specific calibration is required or not, also needs further evaluation to assess the applicability of the method.

The SFR method is a promising tool for assessing growth and biomass by proximal remote sensing, whether it is in a closed growth room or semi-closed greenhouse. It might also be applicable in an open field environment. The method is rather simple, fast and does not necessarily require any expensive equipment, e.g. replacing the spectrometers with photodiodes and filters. It may be suitable for precision farming, helping growers to improve management practices e.g. by analyzing growth trajectories and comparing growth cycles. In combination with controlled supplemental lighting it might be possible to adjust production rate to production demand automatically using this method in a greenhouse environment.

To our knowledge, the strong link between SFR and biomass, both in a closed environment and greenhouse setting, has not so clearly been demonstrated on canopy level before. At least not using such simple active measurement method, with the potential of being applicable for commercial growers.

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