Laser-induced chlorophyll fluorescence sensing to determine biomass and nitrogen uptake of winter wheat under controlled environment and field conditions

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Abstract

The relationship between laser-induced chlorophyll fluorescence intensity (690 nm and 730 nm), ratio F690/F730 and nitrogen supply in winter wheat was characterized using two different sensors (growth chamber and field sensor). In the field, chlorophyll fluorescence was measured at a distance of approximately 3.3 m from the canopy and the sensed area was approximately 6-7 m^2. The fluorescence ratio F690/F730 was inversely correlated with N content and uptake and dry shoot biomass. Shoot dry biomass could be determined by means of biomass index measurements independent of leaf chlorophyll content. The results indicated that nitrogen uptake and biomass can be reliably detected through chlorophyll fluorescence measurements under field and controlled growing conditions.

Keywords: chlorophyll fluorescence, nitrogen, sensor, laser, site-specific nitrogen fertilization

Introduction

In most cases, the application of N fertilizers is carried out without taking into consideration soil and crop spatial variability. Thus, a single N rate based on the average needs of the field as a whole is applied. On the other hand, many fields consist of two or more soil types with different N supply requirements (Carr *et al.*, 1991), requiring different fertilizer management for economically and ecologically reasonable yields. As a consequence of the uniform N management, some sites within the field may be under- or over-fertilized.
Site-specific crop management approaches have been designed to optimise agriculture production by managing crop and soil taking into account the different conditions found in each field. Map- and sensor-based approaches are the basic methods of implementing site-specific management for the variable-rate application of crop inputs.
Sensor-based methods use data acquired in real-time to control site-specific field operations "on-the-go". Such systems can collect data about the current plant growth at a high spatial resolution, enabling the detection of nutrient needs in real-time. With regard to nitrogen fertilization, the question that arises is how the in-field spatial variability in N status of plants can be determined. The measurement of reflected radiation is already being applied to detect N supply differences under field conditions using tractor-mounted systems as e.g. "Yara N-sensor" and "GreenSeeker". Reflectance values may be, however, influenced by soil moisture, row spacing, soil color, time of day and cloudiness, as well as canopy architecture, measuring angle, solar zenith, and variety (Raun *et al.*, 1998). The best reflectance wavelengths for evaluating N status and biomass may change with sampling date, possibly due to differences in soil cover by the crop in different growth stages (Osborne *et al.*, 2002) or in sun position (Raun *et al.*, 1998). Moreover, passive reflectance signatures may be difficult to interpret, since a mixed signal of plant and soil reflectance is detected.

One technique to monitor the nutritional status of plants by means of non-destructive and proximal remote measurements is based on the evaluation of the fluorescence of plant pigments like chlorophyll (Lichtenthaler, 1990, Bredemeier, 2005). This optical method is based on the fact that plant pigments show a typical photon emission termed fluorescence when excited with a natural light source (sun) or a laser beam. Laser-induced chlorophyll fluorescence can be defined as the optical emission from chlorophyll molecules that have been excited to a higher energy level by absorption of electromagnetic radiation.
The chlorophyll a fluorescence spectra of the upper leaf side exhibits 2 fluorescence maxima: one near 690 nm and a second one in the spectral region around 730-740 nm (Buschmann *et al.*, 1994, Lichtenthaler & Rinderle, 1988). The central role played by nitrogen in chlorophyll synthesis suggests that a deficiency in this nutrient in the plant could be detected on the basis of changes in the plant's fluorescence spectra (Lichtenthaler & Rinderle, 1988).
From this point of view, experiments were carried out under controlled environmental and field conditions using two different systems to measure laser-induced chlorophyll fluorescence, namely an innovative tractor-mounted and a hand-held fluorescence sensor. The objective of this work was to evaluate the reliability of proximal remote sensing measurements of laser-induced chlorophyll fluorescence to determine chlorophyll and nitrogen content, as well as biomass production in growth chamber- and field-grown winter wheat.

Material and methods

Growth chamber experiment

Wheat (*Triticum aestivum* L.) plants were grown at a density of 12 plants per pot (8 l volume) filled with a loamy soil. *In vivo* laser-induced fluorescence of chlorophyll was measured using a self-constructed portable fluorescence sensor connected with a portable computer.
The excitation wavelength of the laser device (laser diode) was 640 nm, with power output of 15 mW and modulation of 200 Hz. The sensor detected the chlorophyll fluorescence emitted at 680 nm and 740 nm using photomultiplier detectors and interference filters at a distance of approximately 15 cm between the sensor and the plants. The leaf area illuminated by the laser beam at this distance was about 20 mm^2.
Continuous records of fluorescence of potted plants placed on a rotating plate were used to obtain canopy-averaged measurements. Fluorescence intensity at 680 nm and 740 nm was recorded every 100 ms during continuous illumination with the laser beam. The fluorescence mean value for each pot was the result of single measurements integrated over the canopy. The treatments consisted of different N-levels applied to the plants and different plant densities (13, 16 and 19 plants pot^{-1}). The relative chlorophyll content of the leaf was estimated by using a portable chlorophyll meter (SPAD-502®, Minolta Camera Co., Osaka, Japan). SPAD values have been shown to be well correlated with extractable chlorophyll content especially when expressed on leaf area basis (Bredemeier, 2005).

Field experiments

Field experiments were carried out in 2003 in the tertiary hills of the Bavarian Alps in the southeast of Germany. The experimental site was located in Freising (Germany) (latitude 48°24'N and longitude 11°43'E).
The experiment was arranged in a randomized block design with 4 replications. The plots were 50 m long and 15 m wide. Winter wheat (cv. Ludwig) was sown in October at a seeding rate of 350 seeds m^{-2}. Treatments consisted of different N-fertilization levels applied at different growth stages. The total amount of nitrogen applied varied between 90 and 300 kg N ha^{-1}. For the field

evaluations, a tractor-mounted fluorescence sensor developed by Planto GmbH Company (Leipzig, Germany) connected with a portable computer was used. Sensors were mounted on both sides on the roof of the tractor at a height of 2.8 m from the soil surface (Figure 1).
The sensor used a pulsed laser beam (wavelength 640 nm) as energy source to induce chlorophyll fluorescence emission. The laser-induced chlorophyll fluorescence at 690 nm (F690) and 730 nm (F730) was measured at a distance of approximately 3.3 m between the sensor and the canopy. The canopy was scanned in an approximately 0.6 m wide strip. Strips of approximately 10 m length were measured.
Also the so called "Biomass index" could be determined. The biomass index is related to the frequency at which green plant parts are hit by the laser beam and varies between 0 and 1. If the beam strikes a green leaf, it excites chlorophyll fluorescence, which is delivered to a photodetector by a spherical mirror. Thus, the contact between the laser beam and a green leaf or other green plant part can be detected. Since the instrument probes the canopy repeatedly, fluorescence values that are lower or higher than some threshold value for a green leaf can be eliminated from the data set, so that green leaves can be selectively distinguished from dead leaves or soil.
The fluorescence parameters evaluated, like chlorophyll fluorescence intensity at 690 nm and 730 nm, as well as the ratio between both wavelengths (F690/F730) were compared with destructive harvests of biomass, N content and N uptake.

Ground-truth evaluations

The field experiments were carried out with the objective to spatially match destructive ground-truth measurements of biomass and nitrogen content with laser-induced chlorophyll fluorescence measurements. In this sense, biomass and N content were destructively determined exactly in the same area where the chlorophyll fluorescence measurements were done.

Figure 1. Chlorophyll fluorescence sensors mounted on the roof of the tractor.

After the fluorescence measurements were carried out, shoot fresh biomass was determined on the sensed area in all plots. Destructive harvests were done by cutting plants above ground with a green forage chopper with 1.5 m cutting width equipped with a weighing unit. The harvested area was approximately 12 m^2 at all harvest dates. Ground-truth evaluations in the field were done at different growth stages during the growing period of winter wheat, namely at BBCH 30 (end of tillering), BBCH 39 (flag leaf fully unrolled, ligule just visible) and BBCH 65 (full flowering) (BBCH-Monograph, 1997).
After biomass cutting, samples were dried and ground to pass a 1.5 mm screen and nitrogen content was determined with a Macro-N Analyser (N-Analyser, Elementar Analysensysteme GmbH, Hanau, Germany). The nitrogen uptake per area was determined by multiplying dry shoot biomass by N concentration in the shoot. After biomass cutting, the coordinates (longitude and latitude) of each sampled area in the field were determined with a DGPS (differential global positioning system) receiver.

Results and discussion

Growth chamber experiments

Canopy evaluations were carried out at the 9-leaf-stage in plants grown under controlled environmental conditions. Chlorophyll meter readings (SPAD value) and laser-induced chlorophyll fluorescence intensity at 680 nm and 740 nm increased with increasing N supply (Table 1). The increase of fluorescence intensity with N supply was more pronounced for the 740 nm band than for the 680 nm one. The intensity at 740 nm increased approximately 15%, while at 680 nm, it increased only 8% with increasing N availability. Consequently, the ratio between both wavelengths decreased with N applied (Table 1).

Table 1. Chlorophyll meter reading (SPAD value), laser-induced chlorophyll fluorescence intensity at 680 nm and 740 nm and chlorophyll fluorescence ratio F680/F740 of wheat at the 9-leaf-stage as affected by N supply and plant density.

N applied kg N ha^{-1}	SPAD value units	Fluorescence intensity		Ratio F680/F740
		680 nm counts	740 nm counts	
no N	48.6 c[(1)]	652.4 b	337.3 b	1.9317 a
40	51.5 b	720.6 a	382.9 a	1.8833 a
80	55.7 a	708.6 a	387.8 a	1.8300 b
Plant density plants pot^{-1}				
13	53.3 a	643.2 c	336.4 c	1.9117[ns]
16	51.6 b	687.1 b	367.6 b	1.8733
19	50.8 b	750.7 a	404.0 a	1.8600

[1]Means followed by the same letter in the column within the same factor (N applied or plant density) are not significantly different according to Duncan's Test (P=0.05).
[ns]not significant

As N supply increased, more biomass and more leaves were produced. Consequently, more fluorescence was emitted and detected by the sensor. As shown in Table 1, leaf fluorescence emission significantly increased with N fertilization. Our observation is in agreement with results reported recently by Corp *et al.* (2003), who verified an increase of fluorescence emission with N fertilization across all fluorescence bands evaluated, namely blue, green, red and far-red.

The fluorescence emission was also affected by plant density. As plant density increased, more fluorescence at both wavelengths was emitted and detected (Table 1). The increase was slightly larger for the 740 nm fluorescence band (+20.1%) than for the 680 nm band (+16.7%), leading to a small but not significant decrease of the ratio F680/F740 with increasing plant density (Table 1).

Significant correlations were observed at the 9-leaf-stage between laser-induced chlorophyll fluorescence emission intensity at 680 nm and 740 nm and shoot biomass production. Laser-induced chlorophyll fluorescence emission increased at both wavelengths with increasing shoot dry biomass (Figure 2A). Fluorescence intensity at 680 nm increased approximately 31.6% and at 740 nm approximately 43.3% with increasing shoot biomass from 5.2 up to 8.9 g pot^{-1} (Figure 2A). Due to the larger increment for the 740 nm fluorescence band than for the 680 nm one, the ratio F680/F740 decreased with shoot biomass (Figure 2B). The correlation between the ratio F680/F740 and shoot biomass was highly significant ($P<0.001$).

The results showed that N-fertilization levels, biomass production and chlorophyll content (SPAD value) could be detected by means of laser-induced chlorophyll fluorescence intensity and ratio F680/F740 evaluations at the canopy level. The intensity of the 680 nm fluorescence band as well

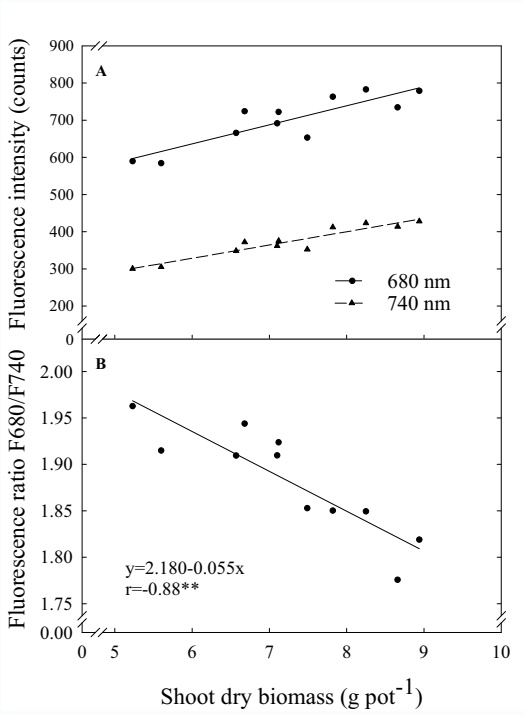

Figure 2. Laser-induced chlorophyll fluorescence intensity at 680 nm and 740 nm (A) and chlorophyll fluorescence ratio F680/F740 (B) as a function of shoot dry biomass for wheat at the 9-leaf-stage. Each data point represents a measurement on a single pot. Lines represent fitting regression curves (y=328.1+51.3x, r=0.87** for 680 nm and y=114.0+35.7x, r=0.95** for 740 nm, *n*=11).

as of the 740 nm band increased with increasing N fertilization and shoot dry biomass. However, our evaluations indicated that this increase was relatively larger at 740 nm than at 680 nm. The higher increase at 740 nm than at 680 nm may be indicating that part of the fluorescence emitted at 680 nm was reabsorbed by photosynthetic pigments within the leaf, since absorption and fluorescence emission spectra of chlorophyll overlap between 660 and 690 nm. The reabsorption caused a decrease of the intensity of the 680 nm fluorescence band. On the other hand, the 740 nm fluorescence band was less affected by the reabsorption phenomena, since light at wavelengths greater than 700 nm is little absorbed by chlorophylls.

As a consequence of the reabsorption of the 680 nm fluorescence band, the fluorescence ratio F680/F740 decreased with increasing shoot biomass and chlorophyll content. The fact that fluorescence ratio F680/F740 was also well correlated with shoot biomass indicated that the fluorescence intensity of single bands and the ratio F680/F740 could be influenced by chlorophyll content as well as by biomass quantity.

Field experiments

Chlorophyll fluorescence measurements and ground-truth evaluations were performed three times during the growing period of winter wheat in 2003, namely at May 6 (BBCH 30), at May 19 (BBCH 39) and at June 5, 2003 (BBCH 65).

Shoot dry biomass and chlorophyll fluorescence variables were well correlated across different developmental stages. Fluorescence intensity at 690 nm and 730 nm increased with increasing shoot dry biomass across all evaluations.

Higher correlations were found between shoot dry biomass and fluorescence intensity at 730 nm than at 690 nm. This may be explained by the fact that fluorescence at 730 nm is little affected by fluorescence reabsorption by chlorophylls that takes place within the leaf. Figure 3 shows the relationship between fluorescence ratio F690/F730, shoot dry biomass and N uptake across different developmental stages. At BBCH 39 and BBCH 69, relationships were better described by power functions. On the other hand, relationships were linear at BBCH 30 (Figure 3). According to our results, it was not possible to fit a single regression curve for all evaluations together. Relationships between fluorescence ratio F690/F730 to shoot dry biomass and N uptake were specific for each developmental stage and could not be extrapolated for other stages, as shown in Figure 3.

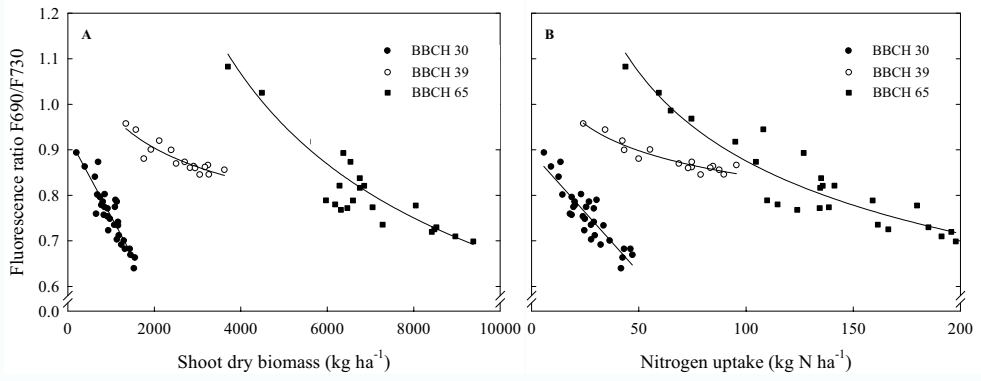

Figure 3. Laser-induced chlorophyll fluorescence ratio F690/F730 as a function of shoot dry biomass (A) and N uptake (B) for winter wheat at different developmental stages in 2003. Lines represent fitting regression curves.

The same behaviour was observed for the relationship between biomass index, shoot dry biomass and N uptake (Figure 4). As shown in Figure 4, it was not possible to fit one single regression curve across all evaluations. The maximum biomass index observed was approximately 0.97. At BBCH 39 and 65, regressions were curvilinear, indicating that the relationship tends to a plateau when biomass index is higher than approximately 0.90 (Figure 4). On the other hand, relationships were linear at BBCH 30. The decrease of biomass index at BBCH 65 is probably related to the higher contribution of spikes and senescent leaves to the total signal detected at this developmental stage (full flowering).

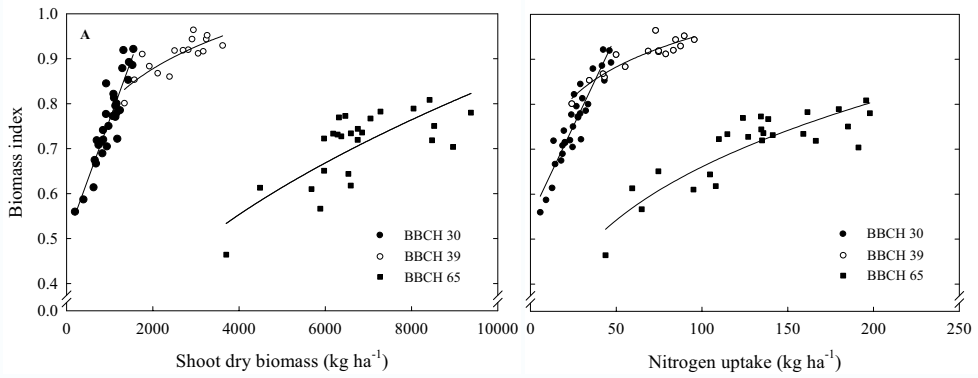

Figure 4. Biomass index as a function of shoot dry biomass (A) and N uptake (B) for winter wheat at different developmental stages in 2003. Lines represent fitting regression curves.

Conclusions

Nitrogen uptake and shoot biomass could be detected by means of chlorophyll fluorescence measurements. In contrast to point data measurements, the establishment of scanning field fluorescence sensors opens new possibilities for N status and biomass measurements. Since the signal comes only from green plant parts, it has a very low background and is little affected by soil reflectance. This feature of chlorophyll fluorescence measurements represents a great advantage compared with reflectance measurements, since reflectance varies greatly depending on light intensity and on the contribution of the soil reflectance to the total signal detected.

Based on these evaluations, it will be possible to monitor on-line the spatial variation of the N-status and biomass production of canopies. The "on-the-go" obtained information can be combined with a fertilizing algorithm to control the amount of N fertilizer being applied. The use of this technique is possible at different developmental stages with the same accuracy, even when the leaf area index is low (as e.g. at the first N dressing). It is expected that this system can be operated in nearly all weather situations, even in the night, with the same accuracy.

References

BBCH-Monograph. 1997. Growth stages of mono- and dicotyledonous plants. Blackwell Wissenschafts-Verlag, Vienna, Austria.

Bredemeier, C. 2005. Laser-induced chlorophyll fluorescence sensing as a tool for site-specific nitrogen fertilization - evaluation under controlled environmental and field conditions in wheat and maize. Shaker Verlag, Aachen, Germany.

Buschmann, C., Nagel, E., Szabó, K. and Kocsányi, L. 1994. Spectrometer for fast measurement of *in vivo* reflectance, absorptance, and fluorescence in the visible and near-infrared. Remote Sensing of Environment 48 18-24.

Carr, P.M., Carlson, G.R., Jacobsen, J.S., Nielsen, G.A. and Skogley, E.O. 1991. Farming soils, not fields: a strategy for increasing fertilizer profitability. Journal of Productive Agriculture 4 51-61.

Corp, L.A., McMurtrey, J.E., Middleton, E.M., Mulchi, C.L., Chappelle, E.W. and Daughtry, C.S.T. 2003. Fluorescence sensing systems: *In vivo* detection of biophysical variations in field corn due to nitrogen supply. Remote Sensing of Environment 86 470-479.

Lichtenthaler, H.K., Hak, R., Rinderle, U. 1990. The chlorophyll fluorescence ratio F690/F730 in leaves of different chlorophyll content. Photosynthesis Research 25 295-298.

Lichtenthaler, H.K. and Rinderle, U. 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. CRC Critical Reviews in Analytical Chemistry 19 (Suppl. 1) 29-85.

Osborne, S.L., Schepers, J.S., Francis, D.D., Schlemmer, M.R. 2002. Detection of phosphorus and nitrogen deficiencies in corn using spectral radiance measurements. Agronomy Journal 94 1215-1221.

Raun, W.R., Johnson, G.V., Solie, J.B., Stone, M.L., Philips, S.B., Dennis, J.L., Cossey, D.A., LaRuffa, J.M., Thomason, W.E., Lukina, E.V. 1998. Sensor based nutrient management. In: Intensive Wheat Management Conference, Denver, 1998. Potash & Phosphate Institute, Brookings, USA. p.18-24.