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# Improved nitrogen retrievals with airborne-derived fluorescence and plant traits quantified from VNIR-SWIR hyperspectral imagery in the context of precision agriculture

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ABSTRACT

In semi-arid conditions, nitrogen (N) is the main limiting factor of crop yield after water, and its accurate quantification remains essential. Recent studies have demonstrated that solar-induced chlorophyll fluorescence (SIF) quantified from hyperspectral imagery is a reliable indicator of photosynthetic activity in the context of precision agriculture and for early stress detection purposes. The role of fluorescence might be critical to our understanding of N levels due to its link with photosynthesis and the maximum rate of carboxylation (Vcmax) under stress. The research presented here aimed to assess the contribution played by airborne-retrieved solar-induced chlorophyll fluorescence (SIF) to the retrieval of N under irrigated and rainfed Mediterranean conditions. The study was carried out at three field sites used for wheat phenotyping purposes in Southern Spain during the 2015 and 2016 growing seasons. Airborne campaigns acquired imagery with two hyperspectral cameras covering the 400–850 nm (20 cm resolution) and 950–1750 nm (50 cm resolution) spectral regions. The performance of multiple regression models built for N quantification with and without including the airborne-retrieved SIF was compared with the performance of models built with plant traits estimated by model inversion, and also with standard approaches based on single spectral indices. Results showed that the accuracy of the models for N retrieval increased when chlorophyll fluorescence was included ($r^2_{LOOCV} \geq 0.92$; $p < 0.0005$) as compared to models only built with chlorophyll a + b ($C_{ab}$), dry matter ($C_m$) and equivalent water thickness ($C_w$) plant traits ($r^2_{LOOCV}$ ranged from 0.68 to 0.77; $p < 0.005$). Moreover, nitrogen indices (NIs) centered at 1510 nm yielded more reliable agreements with N concentration ($r^2 = 0.69$) than traditional chlorophyll indices (TCARI/OSAVI $r^2 = 0.45$) and structural indices (NDVI $r^2 = 0.57$) calculated in the VNIR region. This work demonstrates that under irrigated and non-irrigated conditions, indicators directly linked with photosynthesis such as chlorophyll fluorescence improves predictions of N concentration.

## 1. Introduction

Nitrogen (N) content plays an important role in the plant life cycle. In most situations, N is the major limiting factor of crop yield after water deficiency, and it is an essential element in plant growth (Lemaire et al., 2008). It is well documented that an adequate N supply is crucial for the maintenance of plant biochemistry quality (Nobel, 2009), and that N deficiency greatly changes the photosynthetic capacity, leading to a decrease in photosynthetic quantum yield and light-saturated photosynthetic rate (Khamis et al., 1990). N management of crops has important economic impacts and environmental implications, although nitrogen overfertilization is widely used by farmers as a form of insurance against uncertain soil fertility (Tremblay et al., 2012). In particular, a higher N supply causes significant effects on the environment. Hence, an adequate N management strategy is needed to guide precision diagnosis of soil status and efficient crop management.

Traditionally, the N concentration is estimated using chemical analyses based on leaf tissue, such as Kjeldahl-digestion and Dumas-combustion, due to their reliability in organic N determination. However, these methods are destructive, time consuming, and need complex analysis. Moreover, traditional N estimates provide only limited information, as sampling is based on only a limited number of sites

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in a given field; they are therefore not suitable for the continuous monitoring of N content in the entire field. For these reasons, remote sensing and, in particular, hyperspectral imagery, can be useful for monitoring spatial and temporal variations in crop N content over large areas (Quemada et al., 2014).

The use of simple empirical models that incorporate hyperspectral reflectance indices is still the dominant method used to estimate N (Ferwerda et al., 2005; Stroppiana et al., 2009; Herrmann et al., 2010; Wang et al., 2012; Li et al., 2014; Mahajan et al., 2016). Several studies have shown improvements in canopy N quantifications using reflectance bands in the near infrared (NIR) and in the short-wave infrared (SWIR) regions (Kokaly, 1999; Ferwerda et al., 2005; Herrmann et al., 2010; Pimstein et al., 2011; Gnyp et al., 2014; Mahajan et al., 2014), especially when indices calculated from wavelengths centered at 850 and 1510 nm are used, as described in detail by Herrmann et al. (2010). Serrano et al. (2002) also showed that the combination of the 1510 nm and 1680 nm spectral regions was sensitive to N concentration in green biomass. Nevertheless, and despite the successful empirical relationships, nitrogen estimation at the canopy level from remote sensing requires appropriate modelling strategies due to the large contribution of structural and shadow effects to canopy reflectance (Zarco-Tejada et al., 2005). On the other hand, radiative transfer models offer advantages compared to index-based empirical models regarding robustness and transferability (Jacquemoud and Baret, 1990; Zarco-Tejada et al., 2004; Schlerf and Atzberger, 2006; Wang et al., 2015), and these have been widely proposed as a method for retrieving chlorophyll content, dry matter, and water content from remote sensing data (Clevers and Kooistra, 2012; Jacquemoud and Baret, 1990; Zarco-Tejada et al., 2004). In this context, recent studies have evaluated the estimation of leaf N content using models built with leaf and canopy biophysical parameters retrieved by inversion (e.g. Wang et al., 2015), and these have yielded reasonable success ($r^2 = 0.58$).

In recent years, the quantification of chlorophyll fluorescence has attracted increasing attention in the context of global monitoring of crop physiology and vegetation functioning, and this method can offer improvements on the estimation of N status (Tremblay et al., 2012). Chlorophyll fluorescence is generally considered as a direct proxy for electron transport rate and hence photosynthetic activity (Genty et al., 1989; Weis and Berry, 1987). The leaf-level maximum carboxylation rate (Vcmax; $\mu mol\, CO_2\, m^{-2}\, s^{-1}$) is closely related to the chlorophyll content at leaf scale (Croft et al., 2017; Houborg et al., 2013) and with solar-induced chlorophyll fluorescence (SIF) (Rascher et al., 2015; Yang et al., 2015). In this regard, SIF can be considered as a direct link with Vcmax through its strong connexion to chlorophyll content and photosynthetic activity (Walker et al., 2014). In fact, recent studies have demonstrated the link between chlorophyll fluorescence and photosynthetic activity at leaf and canopy levels (see e.g. Zarco-Tejada et al., 2013, 2016; Cendrero-Mateo et al., 2016). The rationale is based on the dependence of chlorophyll fluorescence emissions on chlorophyll concentration and photosystem I (PSI) and II (*Psi*I) efficiency (Lichtenthaler et al., 1996). It is well documented that N deficiency affects *Psi*I photochemistry, lowering the quantum yield electron transport, the photochemical efficiency, and therefore the assimilation rate (Lu and Zhang, 2000; Jin et al., 2015).

Crop water status may alter N balance: crop N demand is reduced under drought conditions, as growth rate diminishes (Gonzalez-Dugo et al., 2010). In arid and semi-arid environments, the co-limitation between nitrogen and water often reduces crop production which therefore must be considered together (Sadras, 2004). For these reasons, spectral indicators related to the leaf functioning, as chlorophyll fluorescence, is a potentially important candidate for improving the quantification of N concentration using passive remote sensing techniques. The present study aimed to explore the contribution of airborne-retrieved chlorophyll fluorescence to the quantification of N concentration using hyperspectral imagery. Specifically, we evaluated the fluorescence quantification in spring wheat (early sowing) grown under rainfed and irrigated conditions to assess whether they contributed significantly to the retrieval of N concentration in the context of precision agriculture and plant phenotyping experiments.

## 2. Material and methods

### 2.1. Study area

The study was carried out in 2015 and 2016 at three field trial sites for durum wheat (*Triticum turgidum L. var. durum)* and bread wheat (*Triticum aestivum L.)* selection in Southern Spain. The sowing date for all sites was mid-November in the previous year. Regarding fertilization, pest and disease management, all the plots received the same treatment at all trial sites. Fertilization with diammonium phosphate and urea was carried out in early November, while similar amounts of fungicides and pesticides were applied at the early and middle growth stages at all trial sites.

The first trial site was located in Ecija (EC), near Seville, Southern Spain (*37°32′17″N, 5°06′57″W*), which was managed under rainfed conditions in 2015. The experiment was designed with a balanced square lattice design using 300 individual plots (6 × 1.25 m) separated in four blocks, with 150 varieties of durum wheat and 150 of bread wheat. Each cultivar was replicated three times per block (Fig. 1a).

The second site trial was in Carmona (CA), also close to Seville, Southern Spain (*37°30′29″N, 5°34′42″W*) in 2015. The experiment comprised 882 individual plots (7.5 × 1.25 m) divided into two blocks managed under rainfed conditions and one block under irrigated conditions. Each block contained a mixture of varieties of durum and bread wheat, each cultivar replicated three times per block (Fig. 1b).

The third trial site was managed by IFAPA in Santaella (SA), near Cordoba, Southern Spain (*37°31′34″N, 4°50′40″W*) in 2016, where 20 varieties of durum wheat and 20 varieties of bread wheat were replicated three times under irrigated and rainfed conditions (Fig. 1c). The plot size was 15 $m^2$ (10 × 1.5 m).

### 2.2. Field data

In order to assess the physiology and the leaf optical properties of the wheat, a series of leaf-level measurements were made concurrently with the airborne flights at midday (12:00 to 13:00 h local time) at all the trial sites. A summary of field measurements and airborne campaigns at each trial site is shown in Table 1. The wheat growth stage during the flight campaigns refers to the stem length at the time of the first flight in Santaella (SA-1) and grain filling (milking stage) at the time of the flights in EC, CA and the second flight in Santaella (SA-2).

Leaf water potential ($\psi_L$; MPa) was measured using a pressure chamber (Model 600 Pressure Chamber Instrument, PMI Instrument Company, Albany, NY, USA) on two sunlit leaves per plot. Assimilation rate (A; $\mu mol\, m^{-2}\, s^{-1}$) and stomatal conductance (Gs; $mmol\, m^{-2}\, s^{-1}$) were measured using a photosynthesis measurement system (LCDpro-SD, ADC Bioscientific Ltd., Herts, UK) on two sunlit leaves per plot. Steady-state leaf fluorescence yield (Ft) and a SPAD chlorophyll content indicator were measured on 10–15 leaves per plot using a FluorPen FP100 (Photon Systems Instruments, Brno, Czech Republic) and a chlorophyll meter (SPAD-502, Minolta Corp., Ramsey, NJ, USA), respectively. The relationship between chlorophyll concentration and SPAD readings for wheat found by Uddling et al. (2007) was applied to convert SPAD data into chlorophyll content ($\mu g\, cm^{-2}$). Total N concentration was determined by the Kjeldhal method (Kjeldahl, 1883) on 20–25 sunlit leaves sampled per plot. As in the rest of the physiological measurements, a random selection of the sunlit leaves was carried out from the central area of each plot.

### 2.3. Airborne hyperspectral imagery

A hyperspectral imager covering the visible and near-infrared