Short Term Scientific Mission Report

COST Action OPTIMISE: ES1309
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STSM topic: Assessing the link between sun-induced fluorescence, optical properties and photosynthesis in crop canopies.
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STSM type: Regular (from Poland to Germany)
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1. Purpose of the STSM

The primary aim of the STMS was to continue the work carried out during FLEX field campaigns aiming at understanding the link between sun-induced chlorophyll fluorescence (SIF) and plants photosynthesis, and more in the specific, to analyze photosynthetic capacity, SIF and optical properties of the chlorophyll-deficient mutant leaves and canopies in comparison with a green soybean accession (Eiko). The soybean mutant (Minngold) was recently isolated and cloned at the University of Minnesota (USA). The mutation is the result of a nonsynonymous substitution in a Magnesium chelatase ChlI subunit leading to plants with a "yellow" or "golden" phenotype that have approximately 80% less chlorophyll than the Eiko (Campbell et al., 2014).

The above mentioned primary objective was preceded by training the STSM grant holder in SIF measurements and data processing. The training phase took place through: i) participation in another field campaign aiming at understanding how SIF changes within the vertical profile of corn canopies (Zea mays L., Ricardinho variety) grown at two different densities (normal density - 3 plants/m^2 ; reduced plant density - 1 plant/m^2) (details regarding this field campaign activities can be found in the STSM report of dr Chiara Torresan, reference number: COST-STSM-ES1309-34331) and ii) participation in the laboratory activities aiming at HyScreen imaging system setup and testing.

2. Description of the work carried out during the STSM

Main field experiment (Minngold and Eiko soybean accessions)

At the leaf scale:

- the optical properties of Minngold and Eiko leaves were measured by means of the ASD FieldSpec 3 Hi-Res spectrometer (ASD, Colorado, USA), coupled with a FluoWat leaf clip. This portable leaf clip allowed to measure real leaf reflectance, transmittance (without fluorescence contribution) and fluorescence emission under both artificial (active measurements) and natural light conditions (passive measurements). The fluorescence signal measuring principle is based on cutting off the incoming light spectrum above 650 nm with a short-pass filter, which allows recording only the fluorescence emission (650-850 nm), since in this way the measured signal does not originate from reflection (Van Wittenberghe et al., 2013). Upward and downward steady-state fluorescence (F↑, F↓; when the adaxial leaf side was illuminated) were measured by placing the fiber optic into the upper or lower leaf clip opening, respectively. As fluorescence emission is highly dependent on the intensity of incoming photosynthetically active radiation – PAR_{leaf} (400-700 nm, Wm^{-2}) (Meroni et al., 2009), the F signal was normalized for the absorbed PAR_{leaf} ($APAR_{leaf}=PAR_{leaf}*FAPAR_{leaf}$) during the data processing phase. Incoming PAR_{leaf} was measured as the reflected radiance of a white reference, with and without filter, while leaf reflectance and transmittance integrated over the PAR region were used to derive light absorbance and hence fraction of absorbed radiation ($FAPAR_{leaf}$). In our study, active measurements were carried out by means of two types of artificial LED light sources characterized by different emission spectra (LED1 - producing white light, LED2 – producing solely blue peak). Active measurements were performed at leaves located at three different canopy layers (bottom, middle, top), while passive measurements were conducted on fully developed, sun exposed top-canopy leaves (Image 1);
- six active PAM fluorometers (Moni-PAM system, Heinz Walz GmbH, Effeltrich, Germany) were used to monitor fluorescence parameters of constant areas of: 1) top-canopy leaves (3 repetitions for each accession) on a near-continuous basis (sampling every 15 min) during five days of the field campaign and 2) leaves located at three different heights (bottom, middle, top) within the canopy profile during a chosen day of the field campaign. The core of the active Moni-PAM system is the MONI-

HEAD/485 which delivers measuring and actinic light to the leaf through a window that transmits radiation in the range of 400–750 nm, situated at one end of the cylinder. The same blue LED emits actinic light and saturating flashes as well as measuring light: the LED emission maximum and full width at half maximum is 455 nm and 18 nm, respectively. Measuring pulses to excite modulated fluorescence are provided at frequencies of 5 and 100 Hz for measurements of fluorescence under dark and light conditions, respectively. The ambient light reflected from the Teflon sheet is measured with a PAR sensor incorporated inside the measuring head (Porcar-Castell et al., 2007) (Image 2);

- additionally, Minngold and Eiko leaf samples (discs of 1 cm^2) corresponding to different canopy layers (n=23 for each layer and accession) were collected (and stored in liquid nitrogen) and will be used for chlorophyll and xanthophyll cycle pigments measurement and characterization by UV-VIS spectroscopy (Lichtenthaler and Buschmann, 2001) and high-performance liquid chromatography (Thayer and Bjorkman, 1990), respectively.

At the canopy scale:

- canopy reflectance and SIF were recorded by means of the FLOX system – an innovative commercially available system designed to collect unattended, long-term and continuous field measurements (JB Hyperspectral Devices, Neuss, Germany) (Image 3). The current FLOX system was based on two Ocean Optics radiometers (the HR4000 operating in the 400–1000 nm spectral range with a FWHM of 1 nm for VIS/NIR reflectance measurements and the QEPro operating in the 650–800 nm spectral range with a FWHM equal to 0.30 nm for measurements of SIF at the two atmospheric oxygen absorption bands - O2B and O2A, at 689 nm and 760 nm respectively) and an optical multiplexer (MPM-2000, Ocean Optics, Dunedin, FL, USA) able to switch between the channel measuring the incident irradiance (through cosine-receptor foreoptics), a down-looking bare fiber (25° FOV) measuring the up-welling vegetation target radiance and a blind channel for spectral dark current measurements. This thermo-regulated system is able to keep a constant temperature.
- moreover, a new automatic hyperspectral imaging system (HyScreen, SPECIM, Finland) was also used to measure canopy-level reflectance (HyScreen_Full operating in the 400–1600 nm spectral range with a target FWHM and spatial resolution of 2 nm and 0.5 mm, respectively) and SIF (HyScreen_Fluo operating in the 680–780 nm

spectral range with a target FWHM and spatial resolution of 0.2 nm and 1 mm, respectively) (Image 4);

- the closed dynamic (non-steady-state flow-through) chamber system consisting of two chambers, transparent and non-transparent (Image 5), was used in order to estimate canopy-scale CO_2 exchange of two soybean accessions during several days of the field campaign (diurnal cycles). The net ecosystem exchange (NEE, μmol m^{-2} s^{-1}) measured with the transparent chamber and the ecosystem respiration (Reco, μmol m^{-2} s^{-1}) measured with the non-transparent chamber were then used to derive the gross ecosystem exchange (GEP, μmol m^{-2} s^{-1}) (Chojnicki et al., 2010);
- fraction of PAR absorbed by the vegetation canopy ($FAPAR_{canopy}$) was measured by means of the SunScan probe (Delta-T Devices Ltd., Cambridge, UK), which is a 1-m long linear quantum sensor containing 64 photodiodes equally spaced along its length. $FAPAR_{canopy}$ (-) was calculated as:

$$FAPAR_{canopy} = \frac{APAR_C}{PAR_C} = \frac{PAR_C - T_C - R_{CS} + R_S}{PAR_C}$$

where $APAR_C$ is absorbed incident PAR (PAR_C, μmol m^{-2} s^{-1}), T_c - PAR transmitted through the vegetation canopy (μmol m^{-2} s^{-1}), R_{cs} - PAR reflected from soil and canopy (μmol m^{-2} s^{-1}), R_s - PAR reflected from soil (μmol m^{-2} s^{-1}).

Besides $FAPAR_{canopy}$ measurements, Sunscan probe was used to determine the vertical light distribution profile in the Minngold and Eiko canopies by measuring PAR transmitted at different canopy heights (every 15 cm starting from the ground level) (Image 6). The $FAPAR_{canopy}$ measurements were made between 11:00 a.m and 1:00 p.m on two clear days in 6 randomly chosen plots in both soybean fields (measurements in each of the 6 plots consisted of 4 replicates: 2 with sensor centered on the rows, and 2 with sensor placed in between the rows, thus total number of measurements for each accession was equal to 24) with a Sunscan probe oriented parallel to the plant row direction (plants planted in north–south oriented rows). The measurements of the vertical gradient of PAR within the canopy were made with a Sunscan probe oriented in both directions, parallel and perpendicular to the plant row direction (n=3 for each accession, each canopy layer and each measurement direction), in a selected field points, in which both Minngold and Eiko plants were characterized by the same height;
- in addition, canopy density and leaf area index (LAI) of both soybean accessions were assessed destructively at the end of the field campaign.

3. Description of the main results obtained

Table 1 lists the instruments used during the STSM and the measured parameters:

Instrument	Measurement scale	Measured or calculated parameter
Fluowat	leaf	Ftot, Fmax680, Fmax760, PAR_{leaf}, $FAPAR_{leaf}$, $APAR_{leaf}$, Ftot_yield (Ftot/$APAR_{leaf}$), Fmax680_yield (Fmax680/$APAR_{leaf}$), Fmax760_yield (Fmax760/$APAR_{leaf}$),
Moni-PAM	leaf	Fo, Fm, Fm', Ft ETR, NPQ, PhiPSII, PAR_{leaf}
Suncan	canopy	PAR_C, $FAPAR_{canopy}$, R_{CS}, R_S, $APAR_{canopy}$, T_c-T_{60cm}
FLOX	canopy	solar incoming irradiance, top of the canopy reflectance (in the range 400-1000 nm) and SIF (Ftot, Fmax680, Fmax760)
HyScreen	canopy	solar incoming irradiance, top of the canopy reflectance (in the range 400-1600 nm) and SIF (Fmax680, Fmax760)
Chamber systems	canopy	NEE, Reco, GPP

SYMBOLS and ABBREVIATIONS (if not explained in section 2) :

Ftot - the total fluorescence (integrated fluorescence signal between 650 and 800 nm) ($mWm^{-2}sr^{-1}nm^{-1}$)

Fmax680 - maximum fluorescence at O2A band ($mWm^{-2}sr^{-1}nm^{-1}$)

Fmax760 - maximum fluorescence at O2B band ($msWm^{-2}sr^{-1}nm^{-1}$)

ETR - electron transport rate (ETR=PhiPSII·APARleaf·0.5; 0.5 – is a factor that accounts for the partitioning of energy between PSII and PSI) (µmol m^{-2} s^{-1})

F_0 and F_m - minimal and maximal chlorophyll fluorescence yield from dark-acclimated leaves (r.u)

F_t and F'_m - actual and maximal chlorophyll fluorescence yield from light-exposed leaves (r.u)

PhiPSII - photosystem II operating efficiency in light-exposed leaves, PhiPSII=$(F'_m-F_t)/F'_m$ (-)

NPQ - non-photochemical quenching, NPQ=$(F_m-F'_m)/F'_m$ (-)

3.1 Fluowat

The preliminary data analysis showed considerable differences in the fluorescence yield of leaves located at different canopy layers (visible in both oxygen bands and both accessions, however particularly in Minngold), indicating within canopy chlorophyll content vertical gradient. It is also interesting to note that in the mutant leaves, characterized by significantly lower chlorophyll content compared to the green accession leaves, red fluorescence values were higher than those measured in Eiko leaves, probably due to the smaller reabsorption of red fluorescence by photosynthetic pigments within the leaf layers (Fmax685↑ and Fmax685↓, Figure 1).

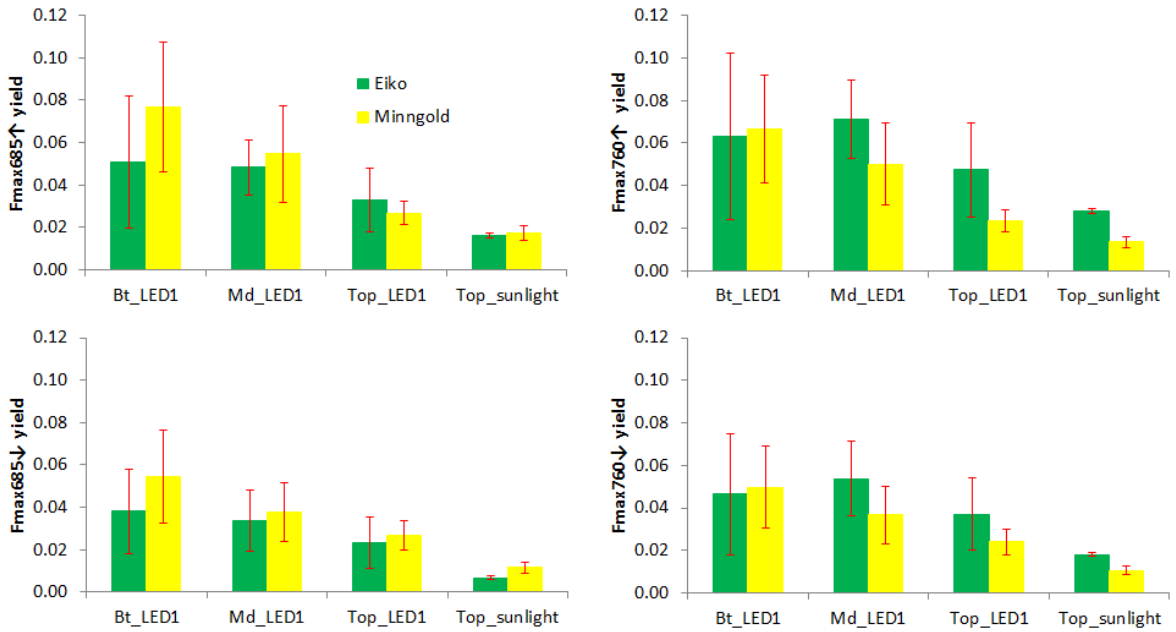

Figure 1. Upward and downward fluorescence yield at the two oxygen bands of Minngold and Eiko soybean leaves ("Bt_LED1", "Md_LED1" and "Top_LED1" refer to bottom, mid- and top-canopy leaves and active measurements (white light source), while "Top_sunlight" refers to the top-canopy leaves measured using the passive approach. Values indicate average of 6 measurements taken on the 25th of July, error bars refer to standard deviation.

3.2 Moni-PAM

The example light response curves of top-leaves photosystem II efficiency - PhiPSII (depicting the fraction of absorbed photons that are used for photochemistry for a light adapted leaf) and electron transport rate - ETR (the actual flux of photons driving photosystem II) for Minngold and Eiko leaves are shown in Figure 2. No significant differences in presented parameters were observed between the two soybean accessions (p value of 0.6 for PhiPSII and 0.32 for ETR, respectively; Welch's t-test). Further analysis of the whole Moni-PAM data set and complementary measurements are needed to corroborate these results.

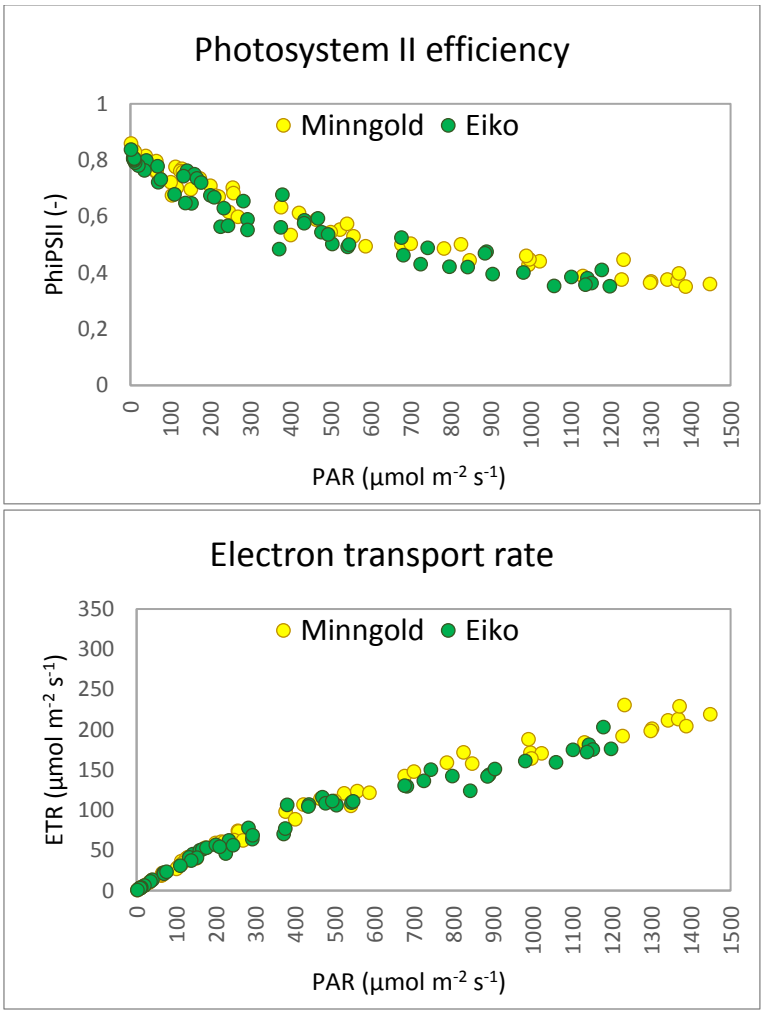

Figure 2. Light response of PhiPSII (upper panel) and ETR (lower panel) for Minngold (yellow circles) and Eiko (green circles) leaves. Values represent the average of 3 leaves. Measurements were taken on the 27th of July.

3.3 FAPAR

The fraction of absorbed photosynthetically active radiation ($FAPAR_{canopy}$) of Eiko soybean accession was approximately 7% higher than $FAPAR_{canopy}$ of the Minngold chlorophyll deficient mutant (p value <0.001; Welch's t-test, Figure 3).

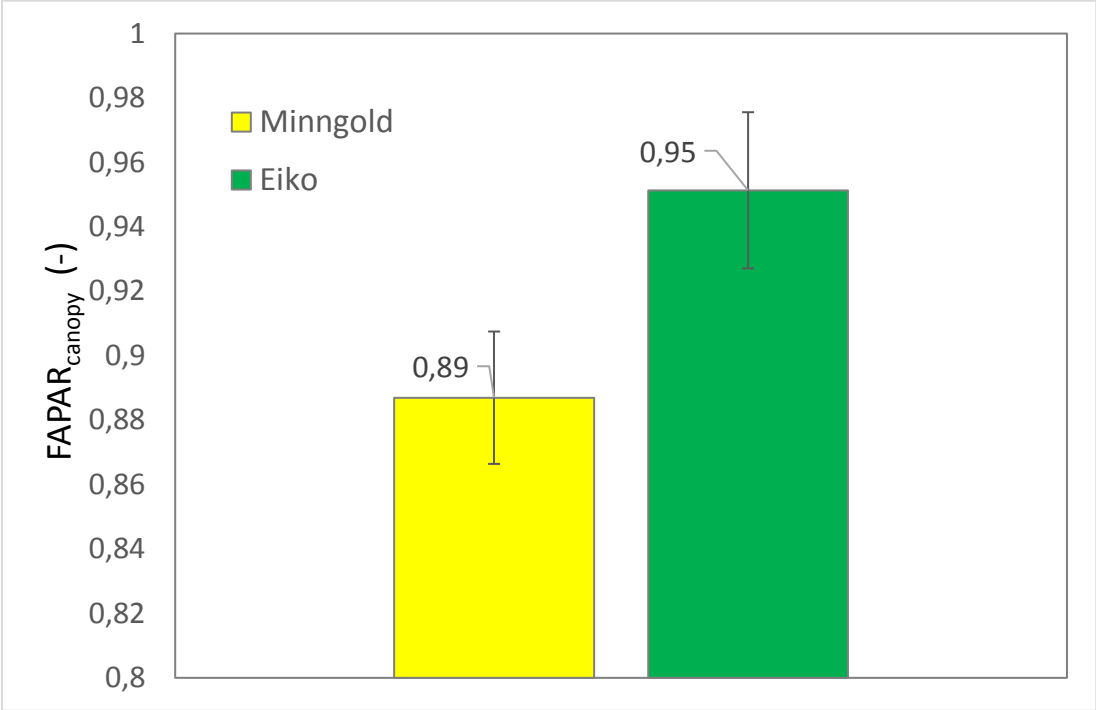

Figure 3. Fraction of absorbed photosynthetically active radiation ($FAPAR_{canopy}$) measured on the 22nd of July in the two investigated soybean accession fields. The values are averages of 24 measurements taken in six randomly chosen field locations. Error bars represent standard deviation.

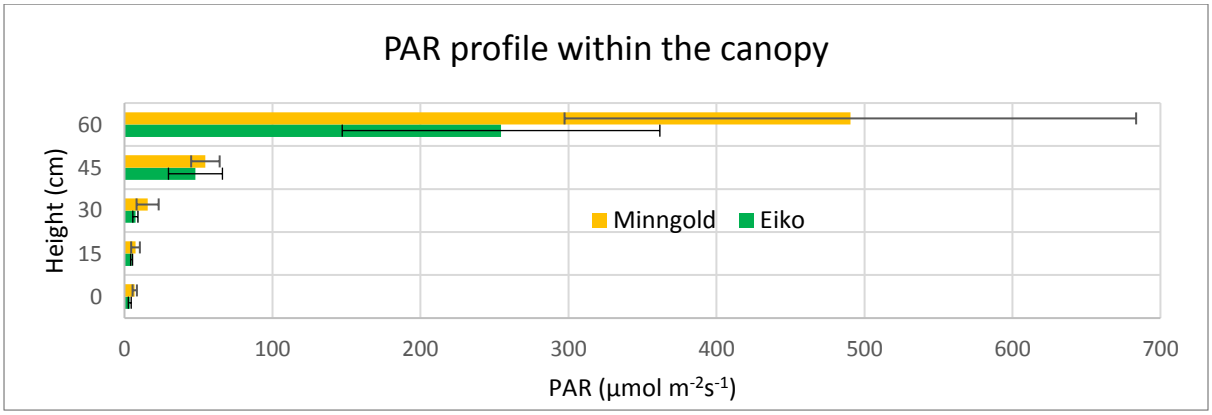

Figure 4. Vertical distribution of incoming photosynthetically active radiation (measured on the 22nd of July) in Minngold and Eiko soybean canopies. The values are averages of 3 measurements, error bars represent standard deviation.

3.4 Chamber CO_2 exchange measurements

Preliminary analysis of chamber data revealed no statistical differences between Minngold and Eiko canopy-scale gross ecosystem production – GPP (calculated on the basis of measured ecosystem respiration - Reco and net ecosystem exchange – NEE) and light use efficiency - LUE (calculated as a ratio between chamber-delivered GPP and Suncan-delivered absorbed photosynthetically active radiation - $APAR_C$) (p value of 0.40 for GPP and 0.56 for ETR, respectively; Welch's t-test, Figure 5).

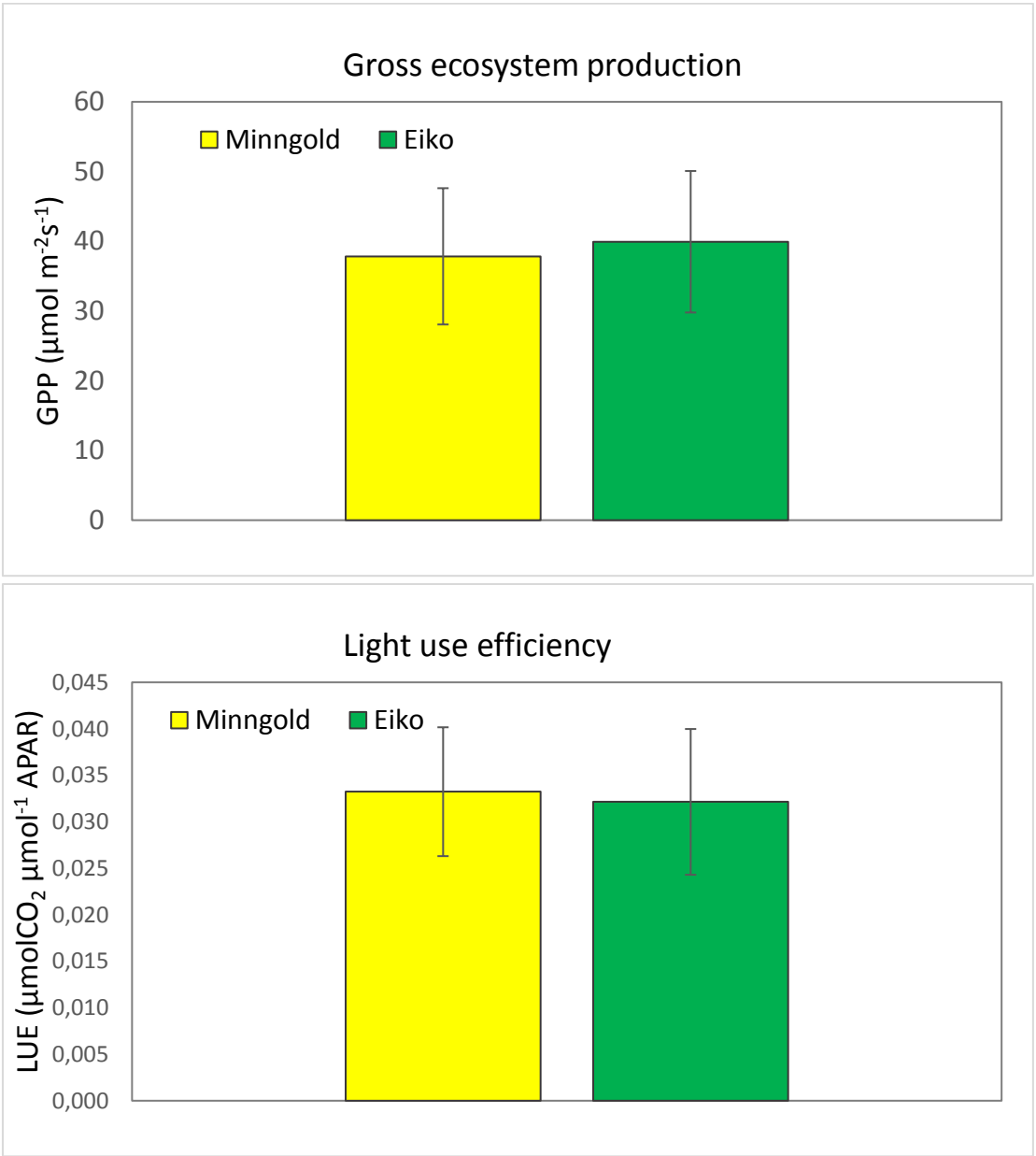

Figure 5. Gross ecosystem production (GEP) and light use efficiency (LUE) of Minngold and Eiko canopies. The values are averages of 32 measurements performed on the 21st and 22nd of July, error bars represent standard deviation.

3.5 Future collaboration with the host institution

Future collaboration with the Host institution will concern processing and analysis of the large and complex dataset collected with various instruments and at different observation scales during the STSM.

3.6 Foreseen publications/articles resulting from the STSM

The work carried out during this STSM will contribute (via oral or poster presentation) to the ESA's workshop on "Remote Sensing of Fluorescence, Photosynthesis and Vegetation Status", which will be held between 17th and 19th of January 2017 ESA-ESRIN, Frascati, Italy. Moreover, a peer-reviewed publication discussing the obtained results is planned (target journal: Remote Sensing of Environment).

Photographs:

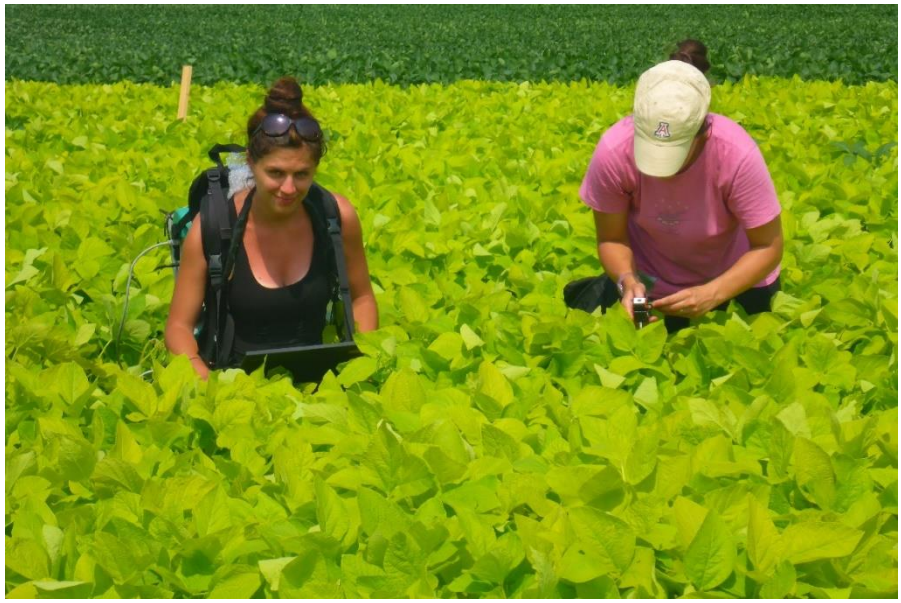

Image 1. Fluowat measurements of top Minngold leaves.

Image 2. Moni-PAM head emitter-detector unit sampling the top leaves of Eiko (left) and Minngold (right) soybean plants.

Image 3. FLOX system installation on a mobile platform.

Image 4. HyScreen system installation.

Image 5. Chamber NEE (left panel) and Reco (right panel) measurements.

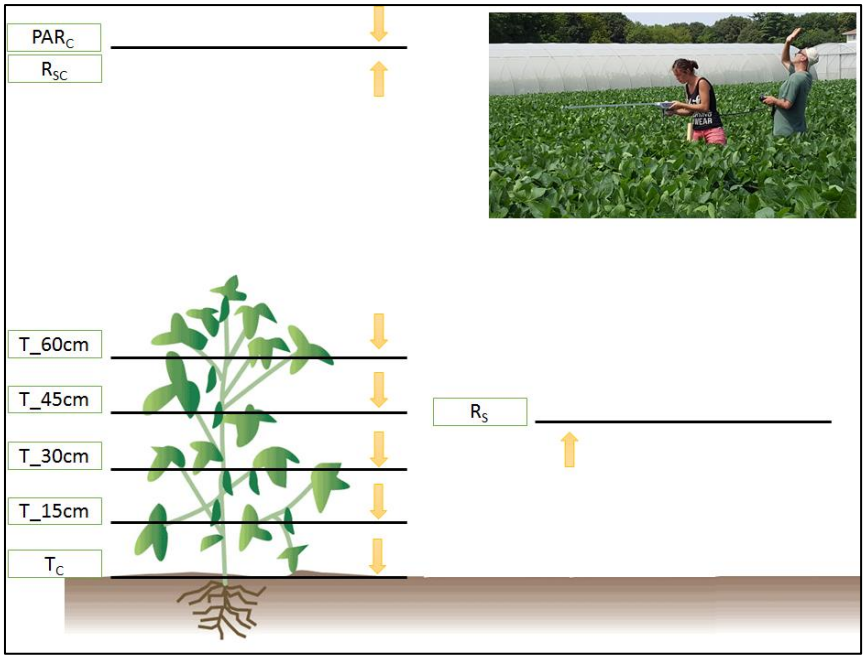

Image 6. Sensor configuration for along-row measurement of i) $FAPAR_{canopy}$ and ii) PAR transmitted at different canopy heights (T_C…T_60cm). Sensors facing upward when measuring downward PAR_C and T, and downward when measuring upward R_S and R_{CS}.

References:

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