Short Term Scientific Mission Report

COST Action OPTIMISE: ES1309

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STSM topic: Understanding the vertical chlorophyll fluorescence gradient in a corn canopy under field conditions

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STSM type: Regular (from Italy **to** Germany**)**

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1. Purpose of the STSM

The STSM at the Forschungszentrum Jülich GmbH was aimed at understanding how the sun-induced chlorophyll fluorescence (SIF) changes from the bottom to the top of a corn (*Zea mays* L.) canopy.
SIF is an electromagnetic signal emitted throughout the red and near-infrared (NIR) spectrum by chlorophyll-a, the primary photosynthetic pigment in green vegetation, in response to the absorption of photosynthetically active radiation (PAR) from the sun. This signal is very low, typically 1%–5% of the reflected radiation in the NIR, and is emitted by the photosynthetic apparatus (Meroni et al., 2009).
SIF can be detected passively or actively. Passive techniques (i.e. sun induced chlorophyll fluorescence), in which the sun light is the excitation light, allow the estimation of the absolute variation in the steady-state chlorophyll fluorescence at leaf, canopy and regional scale (Cendrero-Mateo et al., 2015). In the case of active techniques (i.e. modulated artificial light-induced chlorophyll fluorescence), a constant modulated artificial light is applied as the excitation light.
With the STSM, we combined passive and active measurements to understand vertical profile chlorophyll fluorescence and correlate leaf level measurements with top photosynthesis measurements. In this way we wanted quantify spatial dynamics of SIF within corn canopies and its sensitivity to track patterns of photosynthetic activity originating from the interaction between vegetation structure and incoming radiation as well as variations in plant function.
To reach the purpose of the STSM, field campaign have been performed on corn plot of Ricardinho variety under two different sowing densities, normal density and single plant density.

2. Description of the work carried out during the STSM

2.1 Preparatory activities of the field campaign

During the first two days of the STSM, July the 2nd and 3rd, material preparation, experimental design, and planning activities were carried on.
The first step was a survey in the corn plots located at the “Campus Klein-Altendorf” of the University of Bonn, 70 km far from Jülich (Fig. 1).

Fig. 1 - The yellow polygon shows the area where the corn plots were located (from Google Earth, image dated 10/2/2015), while the yellow star localizes the Campus

All plots of Ricardinho corn were labeled with their specific code, 4 plots between normal density and 4 between single plant density were chosen, 3 plants per plot were selected and labeled, 3 leaves per plant, one in the up, one in the middle and one in the bottom part of the corn stem, were chosen and marked.
In the Table 1, type, level, frequency of measurements, and the instruments selected for our purposes are reported together with the parameters under measuring. The table provides the definition of the acronyms used along the text of the present report.

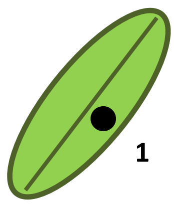
Table 1 - Parameters under measuring, instrument, type, level and frequency of measuring

Parameters	Level	Frequency	Instrument	Type
Reflectance spectrum Vegetation indices: - Normalized Difference Vegetation Index (NDVI) - Simple Ratio Index (SRI) - Photochemical Reflectance Index (PRI)	Leaf	One time per day	PolyPen	Non-Destructive

Parameters	Level	Frequency	Instrument	Type
Full fluorescence spectrum Photosynthetically Active Radiation (PAR) Absorbed Photosynthetically Active Radiation (APAR) Fraction of Absorbed Photosynthetically Active Radiation (fAPAR)	Leaf	One time per day	FluoWat	Non-Destructive
Measurements with dark-acclimated samples: - Minimum fluorescence level excited by very low intensity of measuring light (Fo) - Maximum fluorescence level elicited by a pulse of saturating light (Fm) Measurements with Illuminated samples: - Maximum fluorescence level (Fm') - Steady state fluorescence yield (Fs)	Leaf	Diurnal	Moni-PAM	Non-Destructive
Reflectance spectrum Fluorescence re-emission at O2-B (~687 nm) Fluorescence re-emission at O2-A (~760 nm)	Canopy	One time	FLOX/SIF-Sys	Non-Destructive
Photosynthetically Active Radiation (PAR)	Canopy	One time	SunScan	Non-Destructive
Chlorophyll content Leaf Area Index (leaf that we measured)	Leaf	One time	Lab	Destructive
Leaf Area index per canopy level (upper-middle and middle-bottom)	Canopy	One time	Lab	Destructive

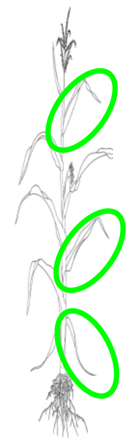
Afterwards, the measurement protocol was specified per each measurement.
In case of Polypen measurements, the number of replicates per sowing density was defined as illustrated in the Table 2.

Table 2 - Measurement protocol for PolyPen

Density	**# plots**	**# plants**	**# level of leaf in the plant**	**# of position in the leaf**	**Total # of measurements**	
Normal	4	3	3 (up - middle - bottom)	1	36	
Single plant	4	3	3 (up - middle - bottom)	1	36	

In case of FluoWat measurements, the protocol was determined as in Table 3.

Table 3 - Measurement protocol for FluoWat

Density	# plots	# plants	# level of leaf in the plant	# of position in the leaf	# of measurements per position	Total # of measure ments	
Normal	4	3	3 (up - middle - bottom)	1	4 (upper leaf with filter, upper leaf, lower leaf, lower leaf with filter)	144	
Single plant	4	3	3 (up - middle - bottom)	1	4 (upper leaf with filter, upper leaf, lower leaf, lower leaf with filter)	144	

In case of MoniPAM system measurements, two corn plants, one from normal density and the other one from the single plant density were chosen. Three leaves were selected in each corn plant, one at the top, one on the middle and one in the lower part of the canopy, and all were accessed using permanently installed elastic wood pole so that both the MONI-head and the leaves would move together in the wind (Fig. 2a, 2b, and 2c). Our protocol measurements defined to record measurements 24 hours over 24 hours for 8 days, from July the 4th to July the 11th. During the day (7 AM to 5 PM) measurements are recorded every 15 minutes, while in the afternoon and night (5 PM to 7 AM) every each 2 hours.

Fig. 2a - The MONI-PAM (Heinz Walz GmbH, Effeltrich, Germany)

Fig. 2b - MONI-head emitter-detector unit in a installation at the top leaf

Fig. 2c - Details of the sample holder with corn leaf

In case of FLOX/SIF-Sys measurements, considering that system is mounted in a sort of bicycle that can drive between the plots and make measurements over the plot, 5 rounds were scheduled to measure top of the canopy fluorescence and reflectance.
In case of SunScan measurement protocol, we decided to measure the PAR at tree heights in the maize canopy: at the top of the canopy (PAR_top_canopy), at the half-canopy-heigt (PAR_middle_canopy), and above the senescent leaves and below the green leaves (PAR_bottom_canopy). We planned to make the measurements in the morning on clear day

with 1 m line quantum sensor (SUNSCAN, Delta, UK) oriented parallel to the plant row direction (plants planted in North - South oriented rows).

2.2 Field campaign activities

Field campaign activities started the 4th July and finished the 11th July. Measurements were carried out as reported in Table 4.

Table 4 - Schedule of field campaign surveys

Instrument	Parameters	Date
PolyPen	Reflectance spectrum Vegetation index (NDVI, SRI, PRI)	5th, 6th, 7th,8th 9th July 2016
FluoWat	Full fluorescence spectrum PAR APAR fAPAR	4th, 5th, 7th,8th 9th July 2016
Moni-PAM	Fo, Fm, Fm', Fs	4th, 5th, 6th, 7th,8th 9th, 10th, 11th July 2016
FLOX/SIF-Sys	Top of canopy reflectance and fluorescence	7th July 2016
SunScan	PAR	7th July 2016
Hand harvest	Chlorophyll content Leaf Area Index (leaf that we measured) Leaf Area index per canopy level (upper-middle and middle-bottom)	11th July 2016

At leaf level, with PolyPen (Photon Systems Instruments Ltd., Brno, Czech Republic) spectral reflectance of an internal light source (Xenon incandescent lamp 380 - 1050 nm) was measured, and use to compute reflectance indices, i.e. NDVI, SRI, and PRI.
At leaf level, a point spectroradiometer (ASD FieldSpec® 3, Analytical Spectral Devices, Boulder, CO, USA) coupled with the FluoWat leaf clip (Alonso et al., 2007; Van Wittenberghe et al., 2013) was used with a spectral range between 350 and 2500 nm and a full width at half maximum (FWHM) of 3 and 10 nm in the 350–1050 and 1050–2500 nm regions, respectively. An artificial lamp, composed with a white and red LED, was used together with the Fluowat to measure at different canopy layers.
Using this portable leaf clip, we measured the whole chlorophyll fluorescence emission spectrum by cutting off the incoming light spectrum with a short-pass filter (< 650 nm). At wavelengths longer than 650 nm, only the SIF emission is recorded, as light in this region is only emitted light. As FluoWat allows measurement of the fluorescence emitted by both sides of the leaf, measurements in the upper and lower side were made. From the ChlF spectrum, SIF at 687 nm and the area between 700 and 715 nm (termed Fw687 and Fw700−715, respectively) were measured.
At leaf level, the MONI-PAM (Heinz Walz GmbH, Effeltrich, Germany) was used to monitor both photochemical and non-photochemical fluorescence quenching during extended time intervals. The MONI-head 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 given at frequencies of 5 and 100 Hz for measurements of fluorescence under dark and light conditions, respectively (Porcar-Castell et al., 2007).

At canopy level, FLOX/SIF-Sys, developed by the Forschungszentrum Jülich GmbH (Burkart et al., 2015), was used to acquire dual beam/field of view (DFOV) data of down-welling and up-welling radiation to measure SIF. Both systems hosts a low cost and small size spectrometer (STS-VIS, Ocean Optics, Inc., Dunedin, US) and uses a bifurcated optical fiber with optical shutters to split the optical signal between two channels: one channel pointing to a white reference panel to measure the down-welling radiant flux and the down-looking channel measuring the radiant flux up welling from the vegetation.
Also at canopy level, SunScan measures incident and transmitted PAR in plant canopy trough a probe which has an array of 64 PAR sensors embedded in 1 m.

3. Description of the main results obtained

3.1 Data processing

Data processing was carried out using R and Matlab software.
First of all starting from the raw data, for each parameter measured in the field, we processed the variables reported in the Table 5.

Table 5 - Processed variables per each measured parameter

Instrument	Measured parameters	Processed variables
PolyPen	Reflectance spectrum Vegetation index (NDVI, SRI, PRI)	
FluoWat	Full fluorescence spectrum PAR APAR fAPAR	Ftot Fmax680 Fmax760 Ftot_yield (Ftot/APAR) Fmax680_yield (Fmax680/APAR) Fmax760_yield (Fmax760/APAR)
Moni-PAM	Fo, Fm, Fm', Fs	Fluorescence Ratio Parameters: - Electron transport rate (ETR) - Effective photochemical quantum yield of Photosystem II (Y(II)) - Non-photochemical quenching (NPQ)
FLOX/SIF-Sys	Reflectance spectrum Fluorescence re-emission at O2-B (~687 nm) Fluorescence re-emission at O2-A (~760 nm)	PAR Fm680 (O2-B) Fm760 (O2-A) Vegetation index (NDVI, SRI, PRI)
SunScan	PAR	APAR fAPAR

3.2 Results from PolyPen measurements

In the plots below (Fig. 3a, 3b), reflectance values averaged in the 5 days of surveys with PolyPen are shows for normal density and single plants sowing conditions with the mean values of standard deviation in a continuous shaded region around the lines.

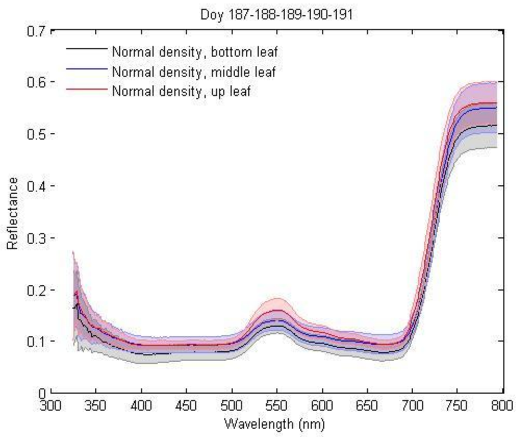


Fig. 3a - Mean reflectance values during 5 days of survey in normal density sowing conditions with the mean values of standard deviation in the shaded region

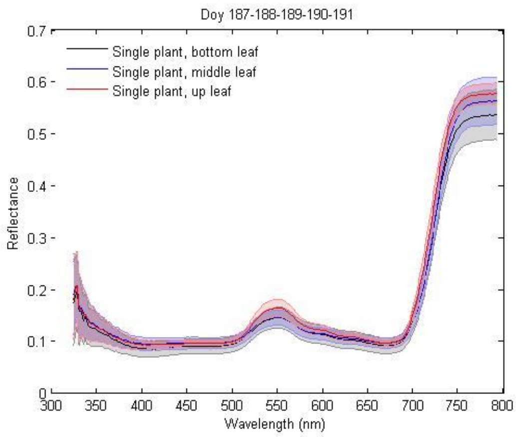


Fig. 3b - Mean reflectance values during 5 days of survey in single plant sowing conditions with the mean values of standard deviation in the shaded region

The results from the paired-sample t-test to compare values of vegetation indices during all 5 days of surveys in two levels of sowing and in different stem position are reported (Table 6, Table 7, and Table 8). For normal density plants significant differences were found in NDVI and SRI between bottom (0.69) > up (0.66) leaves (NDVI, Table 6) and bottom (6.03) > middle (5.51) as well as bottom (6.03) > Up (5.12) (SRI, Table 7). Not statistically differences were found in the case of PRI. In the case of single plants only PRI values between bottom (0.01) < up (0.26) leaves were statistically different (Table 8). From this preliminary analysis we can conclude that sowing density have an effect in the chlorophyll concentration distribution along the different canopy layers. When plants are growing under normal density less light gets to the bottom leaves and as a consequence they increase the concentration of chlorophyll molecules (bottom leaves > SR than middle and Up leaves) to been able to capture more light. On the other hand, the fact that not statistically difference were found in PRI between bottom-middle-up leaves growing under normal sowing density, may indicate that the plants are growing under optimal condition. Enough water and not too strong light (German weather) in available for the plants, thus they do not need to build bigger xanthophyll pool to dissipate the energy that plants cannot use to do photosynthesis. Further analysis need to be done to understand the results obtained for bottom-up single plant leaves.

Table 6 - Paired sample test for NDVI within levels of sowing. ND stands for normal density, SP for single plant, Bt for bottom, Md for middle and Up for up

NDVI	Paired differences			h	p	t	df
	Std. deviation	95% confidence interval of the difference					
		Lower	Upper				
ND, Bt vs Md leaves	0.0332	-0.0319	0.0507	0	0.5608	0.6335	4
ND, Bt vs Up leaves	0.0155	0.0148	0.0532	1	0.0080	4.9058	4
ND, Md vs Up leaves	0.0239	-0.0051	0.0543	0	0.0883	2.2958	4
SP, Bt vs Md leaves	0.0131	0.0148	-0.0178	0	0.8071	-0.2609	4
SP, Bt vs Up leaves	0.0147	-0.0244	0.0121	0	0.4037	0.9328	4
SP, Md vs Up leaves	0.0170	-0.0258	0.0165	0	0.5777	-0.6053	4

Table 7 - Paired sample test for SRI within levels of sowing. ND stands for normal density, SP for single plant, Bt for bottom, Md for middle and Up for up

SRI	Paired differences			h	p	t	df
	Std. deviation	95% confidence interval of the difference					
		Lower	Upper				
ND, Bt vs Md leaves	0.3920	0.0276	1.0010	1	0.0427	2.9338	4
ND, Bt vs Up leaves	0.4627	0.3343	1.4832	1	0.0118	4.3920	4
ND, Md vs Up leaves	0.3746	-0.0706	0.8595	0	0.0781	2.3547	4
SP, Bt vs Md leaves	0.2125	-0.3383	0.1893	0	0.4768	-0.7842	4
SP, Bt vs Up leaves	0.2668	-0.3415	0.3209	0	0.9354	-0.0863	4
SP, Md vs Up leaves	0.1422	-0.1124	0.2408	0	0.3699	1.0094	4

Table 8 - Paired sample test for PRI within levels of sowing. ND stands for normal density, SP for single plant, Bt for bottom, Md for middle and Up for up

PRI	Paired differences			h	p	t	df
	Std. deviation	95% confidence interval of the difference					
		Lower	Upper				
ND, Bt vs Md leaves	0.0094	-0.0114	0.0121	0	0.9384	0.0823	4
ND, Bt vs Up leaves	0.0072	-0.0089	0.0091	0	0.9836	0.0218	4
ND, Md vs Up leaves	0.0028	-0.0038	0.0032	0	0.8379	-0.2183	4
SP, Bt vs Md leaves	0.0228	-0.0437	0.0129	0	0.2054	-1.5107	4
SP, Bt vs Up leaves	0.0037	-0.0123	-0.0032	1	0.0091	-4.7274	4
SP, Md vs Up leaves	0.0196	-0.0166	0.0320	0	0.4296	0.8779	4

3.3 Results from Moni-PAM measurements

In the plots below (Fig. 4a, 4b, 4c), processed variables of a representative day of MONI-PAM measurements are reported for up, middle and bottom leaves of single plant and normal density.

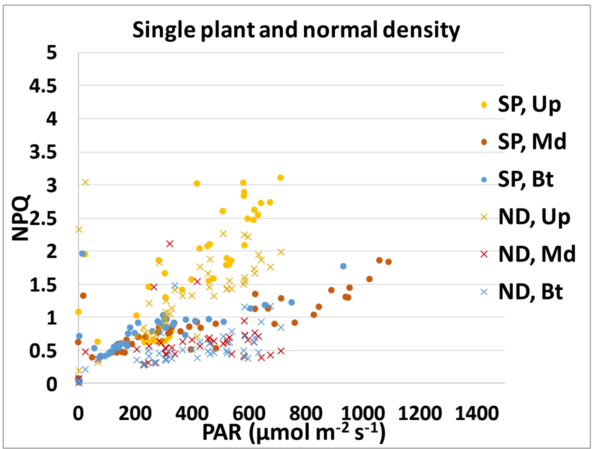


Fig. 4a - NPQ values as a function of PAR irradiance

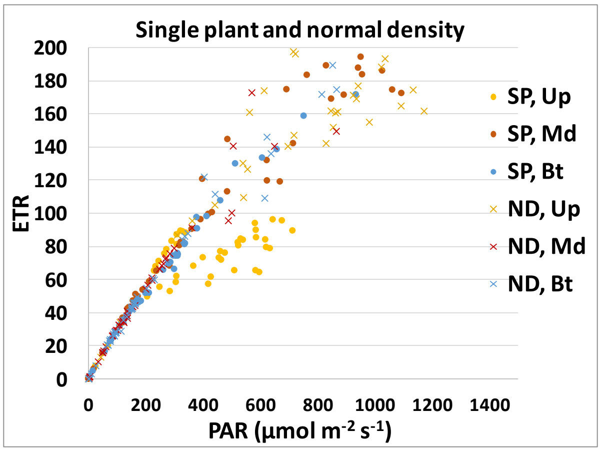


Fig 4b - ETR as a function of PAR irradiance

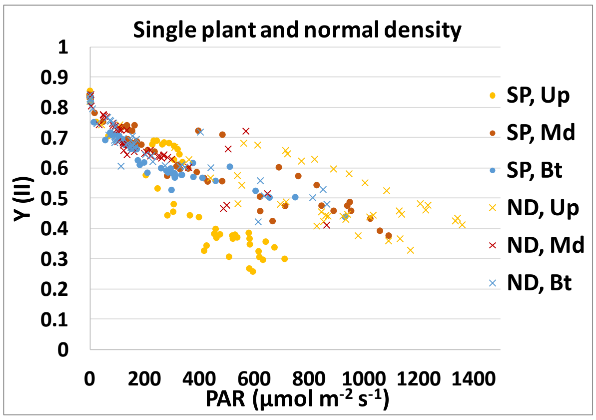


Fig. 4c - Y(II) as a function of PAR irradiance

As general trend, we can highlight that when light increase the differences between the different canopy layers considered increase, both in case of normal density and single plant. Considering in detail some of the parameters measured, if we focus on the non-photochemical quenching, we see that when photosynthetically active radiation increases, leaves in the up part of the canopy take on greater values of NPS with respect to the leaves in the middle part of the corn stem.

Or in the case of effective photochemical quantum yield of Photosystem II, we underline that in the case of single plant the Y(II) is lower in the case of up leaves with respect to the middle and bottom leaves.

Further data processing and analysis are needed to proper understand these results.

3.4 Results from SunScan measurements

In the plots below (Fig. 5a, 5b), the PAR values measured with SunScan within the canopy profile and the fAPAR values at canopy level in the same day of Poli-PAM measurements are reported.

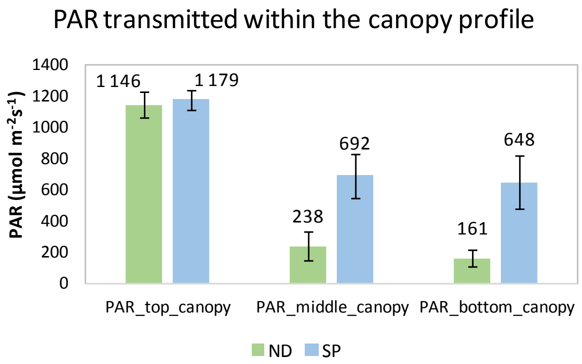


Fig. 5a - The PAR within the canopy profile, in the middle (PAR_middle_canopy) and bottom (PAR_bottom_canopy) portion of the canopy

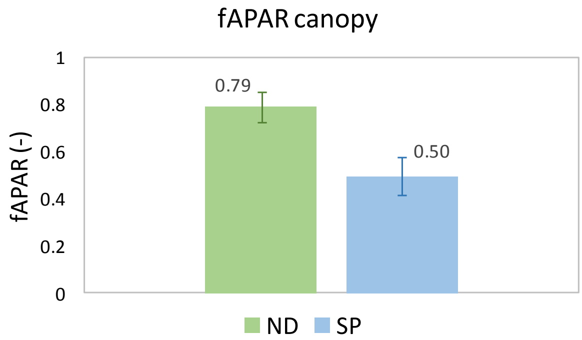


Fig. 5b - The fAPAR at canopy level

As provisional results, we see that there is a certain difference between the PAR in the middle leaves of single corn plant and the PAR in the middle leaves of normal density, being the former higher than the second. The same relation exists in the case of bottom leaves.

The fAPAR, that is the fraction of the incoming solar radiation in the PAR spectral region (fAPAR) absorbed by corn plants in a normal density level of sowing is bigger than that absorbed by corn plants sowed in a lower density, i.e. single plant. Further data processing and analysis are needed to proper understand these results.

3.5 Results from Fluowat measurements

We report processed variables of a representative day of FluoWat measurements, particularly the maximum downward and upward fluorescence yield for 685 and 760 (Fig. 6a, 6b).

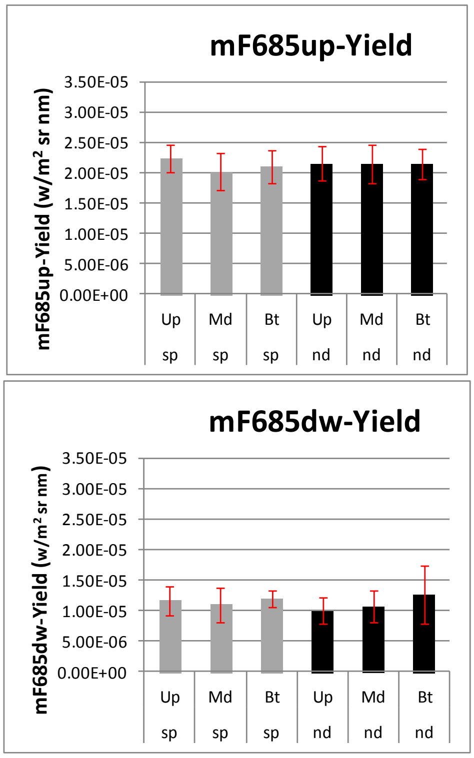


Fig. 6a - The upward and downward fluorescence yield for 685

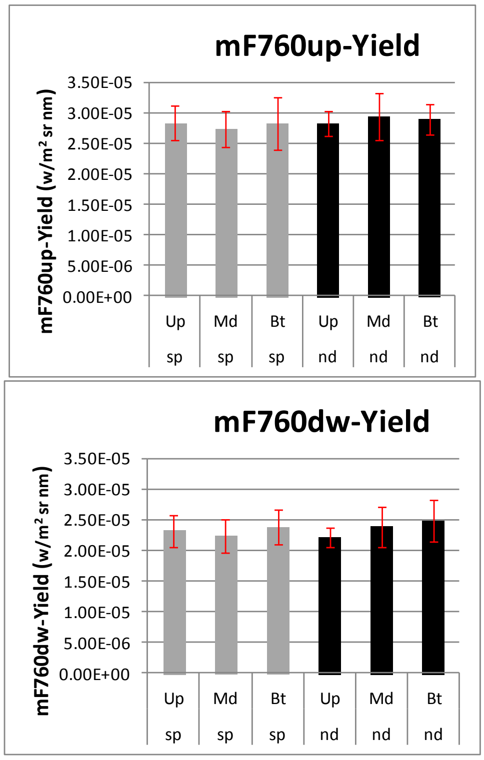


Fig. 6b - The upward and downward fluorescence yield for 760

No differences were observed in 685 and 760 upward fluorescence yield between the bottom-middle-up canopy layers for single and normal density plants. In the case of downward fluorescence yield some difference can be observed between bottom and middle-up leaves. Further data processing and analysis are needed to properly corroborate these observations.

4. Future collaboration with the host institution

At the end of the STSM, we discuss about future collaboration with the host institution:

- investigate the theme of terrestrial sun-induced chlorophyll fluorescence in forest ecosystems;
- considering that the applicant is working in a project aimed at developing a UAV-borne LiDAR system, future collaboration will use this kind of platform for field surveys.

5. Foreseen publications/articles resulting from the STSM

For a publication we still need to analyze FluoWat data for all the days, FLOX data and destructive sample. We will keep working in the data analysis and if conclusive we plan to publish the results.

6. References

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