FLUXPAT-Campaign 2010

Organization of data on the server
Every filename of a zip file consists of the FLUXPAT_campaign_the year.
For each measuring instrument the original data are store in a separate zip file labeled with the appendix***_raw.*** This is due to back-up reasons.
The zip file with the appendix_***V1*** contains all data which can be used for further analysis after a first check. Defective measurements for example are already excluded.
The zip file with the appendix_***V2*** contains one .xls file with all parameter which can be derived from different measurements (read the following of the document).
Values listed in this document are checked and finished analyzed. Data with the flag ***in progress*** have to be checked and analyzed again. Data with ***no data*** had not been measured, or were excluded from analysis due to defective measurements etc.

In-situ measurements
In 2010 the main focus of the plant physiological measurements was the seasonal variation of different parameters in five different species winter wheat, winter barley, rapeseed, sugar beet and maize/corn. Measurements were performed every two weeks.

Table 1: List of In-situ measurements performed in 2010

ID	In-situ measurement	Definition	Measurement Proposed Method
BIOCHEM	Chlorophyll content	Leaf chlorophyll content was quantified at a representative number of leaves within the canopy using the SPAD. For calibration of SPAD data chlorophyll content was determined biochemically by pigment extraction	**leaf-level absorbance measurements in the red and near-infrared (SPAD) and biochemical pigment extraction**
PAM	Efficiency of light reaction (leaf level)	Efficiency of light reactions was determined as $\Delta F/F_m'$ over a representative sample of leaves within the canopy. Additionally electron transport rate (ETR) and non-photochemical protection mechanisms (NPQ) was quantified	**Chlorophyll fluorescence measurements according to the saturating light pulse method (Mini-PAM and PAM-2000)**
	Cardinal points of light response characteristics	Spot measurements of fluorescence parameters were fitted using a photosynthesis model and maximum electron transport rate (ETR_{max}) were determined (definitions: Rascher et al. 2000)	**Fitting of fluorescence parameters to mechanistic photosynthesis model**
GAS	Photosynthetic CO_2 uptake rate and water evapo-transpiration	Rate of photosynthetic CO_2 uptake and rate of H_2O release were quantified by leaf level gas-exchange at a representative number of leaves being exposed to the prevailing range of light intensities	**leaf level gas-exchange (LICOR 6400)**

For the BIOCHEM, and GAS measurements with three to four spots per field were measured. The spots were marked by GPS. Even though for PAM over a 150 single measurements was performed only one finished value of each parameter per field can be derived.

Biochemical Parameters (BIOCHEM.)
Leaf chlorophyll content
The leaf chlorophyll content was measured with the *Chlorophyll Meter SPAD-502* (Figure 1). Observation of changes in chlorophyll content has applications in basic photosynthesis research. The chlorophyll meters illustrate changes in chlorophyll content which can be correlated to plant health and condition. This data can even be used to compliment chlorophyll fluorescence and gas analysis measurements. Chlorophyll has several distinct optical absorbance characteristics that the chlorophyll meters exploits to measure relative

chlorophyll concentration without destructive sampling. Strong absorption bands are present in the blue and red but not in the green or infrared bands, hence the green appearance of a leaf. By measuring the amount of energy absorbed in the red band an estimate of the amount of chlorophyll present in the tissue is possible. Measurements in the infrared band show absorbance due to cellular structure materials. By using this infrared band to quantify bulk leaf absorbance, factors such as leaf thickness can be taken into account in the CCI (Chlorophyll Content Index) value.
The SPAD readings are relative measurements, hence calibration measurements, using laboratory analysis methods, are necessary. Therefore leaf disks were cut with a standardized cork borer, placed in plastic tubes and frozen and stored in liquid-nitrogen.
Leaf pigments were later extracted in laboratory using the method after Lichtenthaler (1987). Additionally separate leaf disks were taken to for chlorophyll analyses independent from the SPAD readings. For each spot three probes were taken and pigments were analyzed in the lab.

Figure 1 Chlorophyll Meter SPAD-502(left), sampling of leaf disks to calibrate SPAD readings (right).

PAM fluorometry (PAM)
Light reaction of photosynthesis was measured using the Miniaturized Fluorescence Yield Analyzer of Walz Inc. The fluorescence signal of chlorophyll *a* can accurately be quantified with this field portable instrument, which can be carried in the different field and that was used to measure photosynthetic activity and non-photochemical energy dissipation processes. Chlorophyll *a* fluorescence was measured using the miniaturized pulse-amplitude modulated photosynthesis yield analyzer (Mini-PAM) of H. Walz (Effeltrich, Germany) with a leaf clip holder described by Bilger, Schreiber & Bock (1995) (Figure 3). Spot measurements of light intensity (λ = 380-710 nm) were taken inside the measuring field by the micro-quantum sensor of the Mini-PAM. Several leafs were dark adapted additionally with Dark Leaf Clips to detect optimal quantum yield. Dark-adapted values (n=30) of optimal quantum yield of PS II (F_v/F_m) were calculated as $F_v/F_m = (F_m - F_0) / F_m$, with F_m being the maximum fluorescence of the dark-adapted leaf when a saturating light pulse of 800 ms duration (intensity ≈ 4000 µmol m^{-2} s^{-1}) was applied. The effective quantum yield of PS II ($\Delta F/F_m'$ (Qeff)) was calculated as $(F_m' - F) / F_m'$, where F is fluorescence yield of the light adapted sample and F_m' is the maximum light-adapted fluorescence yield when a saturating light pulse (as described above) was superimposed on the prevailing environmental light levels. During these measurements

special care was taken not to change the ambient conditions, e.g., the angle of the leaf or shading. Non-photochemical processes (NPQ) were calculated as $(F_m - F_m') / F_m'$. Prior to and just after each measurement, a fluorescence standard was measured, which was used to correct absolute values. The apparent rate of photosynthetic electron transport of PS II (ETR) was obtained as $ETR = \Delta F/F_m' \cdot PPFD \cdot 0.5 \cdot$ Reflection factor, where the factor 0.5 assumes equal excitation of both PS II and PS I; Reflection factor was derived from leaf level absorption measurements using the integrating sphere.
Light within the canopy changed during morning hours and showed patches of varying intensity. Thus, leaves were exposed to rapid changes in PFD of various duration and intensity, which could not be determined analytically. $\Delta F/F_m'$, ETR and NPQ values dynamically adapt primarily to these changes in light intensity, but may also reflect manifold underlying physiological mechanisms. Additional information on characteristic plant parameters of a species, which are not related to the momentary ambient light conditions, but rather to the ontogeny of a leaf and to the range of physiological plasticity of a plant, can be derived from light response curves. Therefore all *ETR* values (n > 150) were fitted over the *PPFD* with an exponential rise to maximum function in order to quantify the maximum electron transport rate (ETR_{max}), which is an indicator of the photosynthetic capacity of the plant canopy (Rascher et al., 2000). To eliminate the dependence of light intensity on *NPQ*, the mean for each 1.5-hour window of all measured *NPQ* values between a *PPFD* of 900 and 1300 µmol m^{-2} s^{-1} was taken to give the non-photochemical quenching parameter at the saturating light intensity of 1100 µmol m^{-2} s^{-1} (NPQ_{1100}). The same analyses step was done for effective quantum yield of PS II ($Q_{eff1100}$) and terminal fluorescence Ft. The therefore derived parameter is called Ft_{1100}.

Figure 2: Mini-PAM which was used to quantify light reactions of photosynthesis in the field. The open leaf clip holder allows a non destructive sampling of a large number of leaves in a canopy under prevailing environmental conditions (left). Additionally several leaves inside the canopy were dark adapted to determine the potential quantum yield.

Table 2: Parameters derived from the Pam measurements.

ID	Parameter	unit	
ETRmax	Maximum electron transport rate at saturating light intensity	$\mu molCO_2 m^{-2} s^{-1}$	
NPQ_{1100}	Non-photochemical quenching at high light intensity	a.u.	
$Qeff_{1100}$	Effective Quantum Yield	a.u.	
Fv/Fm	Potential Quantum Yield	a.u	
Ft_{1100}	Steady state fluorescence at high light intensity	a.u	

Leaf-level gas-exchange (GAS)

A-ci curves for each species were measured. Therefore the leaf temperature and humidity were hold constant at high light condition and CO_2 concentration inside the chamber was regulated according to Table 3. The values marked with an x were excluded from further analyses of the data. From these curves for example Vc_{max} values can be derived.

Table 3: Controlled reference cell CO_2 value for A-ci curves.

Reference cell CO_2 (µmol CO_2 mol^{-1})	
2000	
2000	
1500	
1000	
900	
700	
500	
380	
200	
100	
50	
0	

Leaf-level gas exchange was measured using the LI-6400 (LiCor, Lincoln, NE, USA) (Figure 3.). This is an open measurement system, where air flow was moved through a controlled atmosphere surrounding a plant leaf enclosed in an assimilation chamber. The CO_2 and H_2O exchange was then measured with infrared gas absorbance. The CO_2 level of the air was maintained in a steady state at 390 ppm. The light response curves of the CO_2 assimilation rate (*A*) were measured using the LED light source LI-6400-02B (LiCor, Lincoln, NE, USA). Radiation was set to 2000, 1000, 500, 200, 100, 50, 20, 10 µmol m^{-2} s^{-1} and dark. Air humidity and temperature inside the measuring chamber were adjusted to ambient conditions.

To characterize the potential photosynthetic performance in between different field apparent maximum photosynthetic CO_2 uptake rate (A_{max}) was estimated from each light response curve of gas exchange measurements using an exponential rise to maximum function (Fig. 1). Light curves were only measured for maize in 2010 because with the Aci-curves listed in the protocol above not the maximum carboxylation rate of Rubisco (Vcmax) but only of Phosphoenolpyruvatcarboxylase can be derived. While this is not used as an input parameter for the CLM more benefit can be taken out of Light response curves.

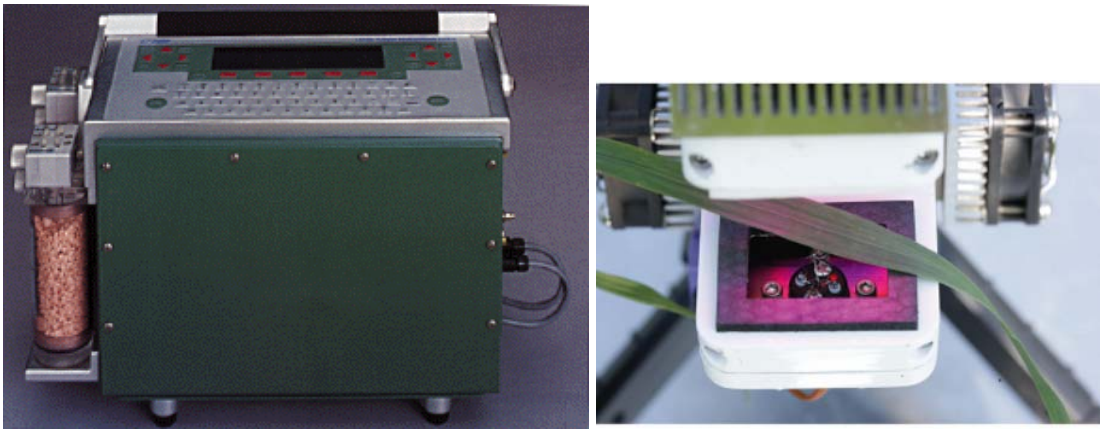

Figure 3: LI-6400 portable gas exchange measurement system.

Table 4: All parameters derived from gas exchange measurements

ID	Parameter	unit	
Amax	maximum photosynthetic CO_2 uptake rate at saturating light intensity	$\mu molCO_2 m^{-2} s^{-1}$	
Rd	Dark respiration	$\mu molCO_2 m^{-2} s^{-1}$	
Vcmax	Maximum carboxylation rate	$\mu molCO_2 m^{-2} s^{-1}$	
mp	Slope of conductance to photosynthesis relationship (ball-berry)		
bp	Minimum leaf conductance /offset of conductance to photosynthesis relationship (ball-berry)	$\mu molCO_2 m^{-2} s^{-1}$	

Location of characterized fields

In Figure 4 and 5 an overview of all characterized field in 2010 is given. In Table 5 an overview of the performed measurements per field is listed

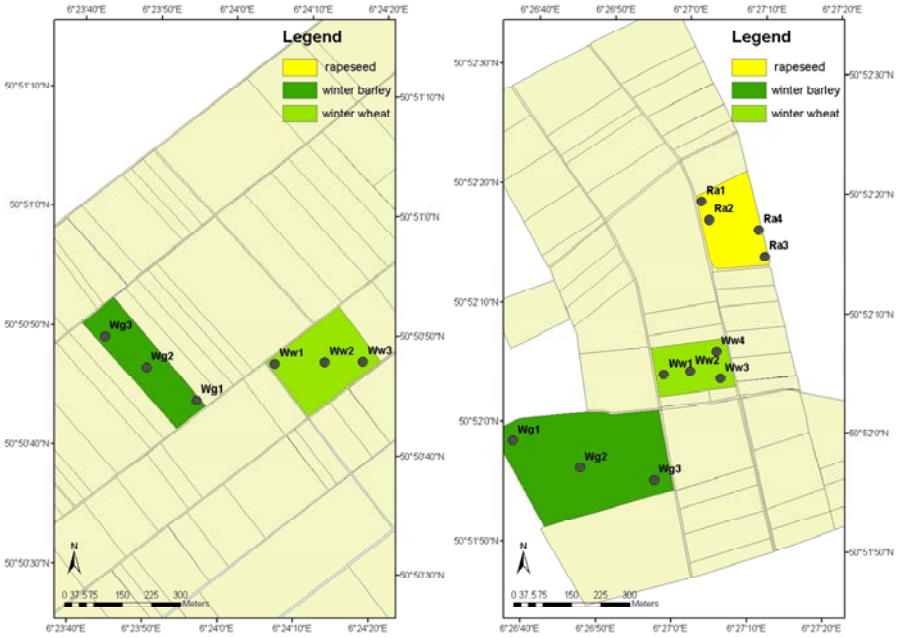

Figure 4: Measurement fields in the area Merken (left) and Selhausen (right); location of the fields (field-id) where in-situ measurements were performed in 2010 on 01.04.10 and 15.04.10.

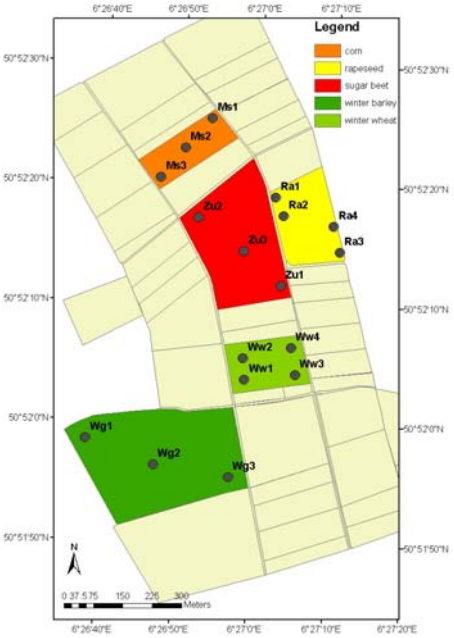

Figure 5: Measurement fields in the area Selhausen; location of the fields (field-id) where in-situ measurements were performed in 2010 since the 29.04.10.

Table 5: In-situ measurements in 2010

DATE	FIELD-ID	PAM	BIOCHEM	GAS
01.04.10	Ra, Ww, Wg		☑	☑
15.04.10	Ra, Ww, Wg	☑	☑	☑
29.04.10	Ra, Ww, Wg	☑	☑	☑
12.05.10	Ra, Ww, Wg		☑	☑
27.05.10	Ra, Ww, Wg	☑	☑	☑
10.06.10	Ra, Ww, Wg, Zu	☑	☑	☑
24.06.10	Ra, Ww, Wg, Zu, Ms	☑	☑	☑
07.07.10	Ww, Zu, Ms	☑	☑	☑
27.07.10	Zu, Ms	☑	☑	☑
06.08.10	Zu, Ms	☑	☑	☑
18.08.10	Zu, Ms	☑	☑	☑
03.09.10	Zu, Ms		☑	☑
17.09.10	Zu, Ms	☑	☑	☑
01.10.10	Zu, Ms	☑	☑	☑
17.10.10	Zu, Ms	☑	☑	☑

Literature:

Lichtenthaler, H.K., 1987. Chlorophylls and Carotenoids - Pigments of Photosynthetic Biomembranes. Methods Enzymol., 148, 350-382.

Rascher, U., Liebig, M. and Luttge, U., 2000. Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. Plant Cell Environ., 23(12), 1397-1405.

Bilger, W., Schreiber, U. and Bock, M., 1995. Determination of the Quantum Efficiency of Photosystem-II and of Nonphotochemical Quenching of Chlorophyll Fluorescence in the Field. Oecologia, 102(4), 425-432.