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Full Length Research Paper

Environmental monitoring of soil conditioner effects on photosynthetic parameters of *Acer campestre* L.

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We evaluated photosynthetic reactions in *Acer campestre* L. in a multi-factorial field experiment near Hodonín, Southern Moravia, Czech Republic. In this experiment, selected plots were amended with three different supplementary soil substances, that is, zeolite, lignite, and hydroabsorbent *Agrisorb*, and were compared with unamended control plots. Microclimatic parameters, that is, temperature, relative humidity, soil moisture, and solar radiation were measured. In the spring of 2008, supplementary soil substances were applied, and in the autumn of the same year, experimental *A. campestre* L. plants were harvested. The basic physiological parameters, chlorophyll content and fluorescence, stomatal conductance, and sap flow were determined in the experimental plants. Determination of the above-mentioned parameters showed higher vitality of plants cultivated on plots supplemented by lignite, followed by zeolite. In comparison with the control plants, the application of lignite led to increase of average chlorophyll content and fluorescence by 37 and 20%, respectively, stomatal conductance by 29%, and sap flow by 44% in plants. In comparison with the control plants, the application of zeolite resulted in the enhancement of average chlorophyll content and fluorescence by 14 and 15%, respectively, stomatal conductance by 16%, and sap flow by 36% in plants. The monitored parameters of plants cultivated on plots supplemented with hydroabsorbent *Agrisorb* were similar to those measured in control plants. In addition, the results obtained are related to actual weather. Correlations between type of the applied supplementary soil substances, soil moisture, and physiological parameters were found. Data of short-time measurement of photosynthesis response correspond with data of long-time measurement of morphological characteristics. The highest height increments were determined in experimental group cultivated under lignite application, followed by zeolite. Statistically insignificant plant height increases were observed in plants cultivated on the soil supplemented with hydroabsorbent *Agrisorb*.

Key words: Abiotic stress, soil conditions, microclimatic data, photosynthetic reactions, morphological characteristic, environmental sensors.

INTRODUCTION

Drought is the most limiting factor for crops in agriculture comparing to other stress factors (Simelton et al., 2009; Yang et al., 2005). It is connected with the reducing plant growth and yields, thus, drought brings economic losses in agriculture (Sisto et al., 2011). Limited water availability in connection with the other factors associated with stress represents the important issue to be solved in agriculture

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(Pekin et al., 2009; Pigott and Pigott, 1993). Drought stress can be reduced using substances that are able to retain the water in soil as supplementary soil substances belonging to such group of compounds (Andry et al., 2009; Shalaby, 1993). They represent one of the possibilities to reduce drought stress and to improve chemical, physical, and biological properties of soils (Bhardwaj et al., 2007; Bouranis et al., 1995). Due to their ability to retain water, they can gradually release water to plants (Beniwal et al., 2010; Gehring and Lewis, 1980; Chen et al., 2004) and, thus, can significantly improve response of plants to drought (Abd El-Rehim et al., 2004; Singels et al., 2010; Zohuriaan-Mehr et al.,2010). Besides agriculture, drought stress is also very important in forestry, where determination of water capacity of soil is important for predicting, which tree species are able to grow under given conditions (Piedallu et al., 2010). Water soil represents the most important factor for plants (Asgarzadeh et al., 2010).

Choi and Moon (Choi and Moon, 2011) developed soil wetting agent using polyoxyethylene nonylphenyl ether and polyoxyethylene castor oil, which increased water soil availability and subsequent growth of experimental plants of *Capsicum annuum* L. cv. Knockwang. Novel nanosuperabsorbent composites based on heteropolysaccharide structure were used and tested by Singh et al. (2010) with possibility to improve water retention in sandy loam soils. There were also investigated substances like acidic and alkaline ash (Stoof et al., 2010). In the case of fly ash, authors accentuated not only its beneficial effect on plants (which must be further investigated), but also end-use of fly ash and its deposition in soils (Yunusa et al., 2010). Wood wastes and other substances originating from human activities are also tested as supplementary soil substances (Venner et al., 2010). Natural substances are intensely investigated not only with focus on water retention, but also on nutrient leaching to maintain soil fertility (Garba et al., 2011). Natural organic substrates compost and vermicompost showed positive effect on plant growth with well-balanced releasing of macronutrients in tests on *Ipomoea aquatica* Forssk (Jouquet et al., 2010). Positive changes of soil supplementation by mulch in carbon, nitrogen, potassium, and phosphorus were recorded in semi-arid regions (Iqbal et al., 2010).

To evaluate effect of supplementary soil substances, determination of markers connected with drought stress is needed. The study of the physiological responses of plants and their connection with stress is, especially in the field conditions, very complicated, mainly because of contemporaneous effect of two or more stress factors (high solar radiation, high surface temperature, increased transpiration, or ion unbalance in soil solution) (Chakraborty and Li, 2010; Jaworski et al., 2010). Interactions between these factors may significantly change and modify character of stress reaction and response comparing to the effects of stress factors individually. The effect of stress factors is mainly restricted to leaves or roots (Perez et al., 2010; Zyalalov, 2004). Laboratory methods and techniques are often time-consuming and usually destructive for the determination of physiological state of plants. Sample may be analysed only one time, so monitoring of changes during vegetation period is practically impossible. For the long-term monitoring of state of plants directly in field conditions, apparatuses for non-destructive measurements are needed. Such instruments are now available and can be used for determination of growth parameters as height and radial increments, which are mainly influenced by stress (Babula et al., 2007; Li et al., 2011; Petrek et al., 2007; Petrek et al., 2005; Supalkova et al., 2008; Supalkova et al., 2007b; Vacek et al., 2004; Vitecek et al., 2005; Vitecek et al., 2004; Vitecek et al., 2007).

In this study, we focused on studying of the effect of supplementary soil substances as zeolite, lignite and hydroabsorbent *Agrisorb* on the growth, development and some physiological parameters of *Acer campestre* L. Non-destructive methods were used for determination of chlorophyll content and chlorophyll fluorescence in the leaves, and for determination of stomatal conductance and sap flow in the whole plants. Moreover, mathematical and statistical evaluations of the obtained data were done. The aim of these measurements and evaluations was to select the most suitable supplementary soil substances applicable on the soil suffering by drought.

MATERIALS AND METHODS

Experimental scheme

In March 2008, three different supplementary soil substances (zeolite, lignite and hydroabsorbent *Agrisorb*) were applied into the soil of the experimental plots located at 48°52′39.654″N, 17°7′49.171″E in the vicinity of Hodonín-Pánov, Southern Moravia, Czech Republic (Figure 1). The plots are located in a locality with degraded soil and low precipitation amounts averaging 569 mm, of rainfall annually and 355 mm in vegetative season. Characterization of soil and individual supplementary soil substances including applied dosage is described in the following sections. The experimental plot without supplementary soil substances application served as control (Figure 1). All experimental variants (including also control plot) were carried out in triplicates. In October 2008, two year old experimental plants of *A. campestre* L. (field maple) were transplanted in the plots (10 per a plot) to study the ability of supplementary soil substances which affect chlorophyll content, chlorophyll fluorescence, stomatal conductance and sap flow. *A. campestre* L. plants were chosen in accordance with following parameters: suitability for given field conditions, autochthony, dynamics of growth, type of root system, size of leaf area, rate of adaptability of plants, and effectiveness of their usage under given conditions. Stress conditions and plants responses to these conditions were monitored by the use of photosynthetic parameters, such as content of chlorophyll and its fluorescence, stomatal conductance, and sap flow during five days (from 21 to 25 August 2010) under the strictly defined one-hour intervals from 9:00 am to 6:00 pm. The obtained data were related to the actual microclimatic conditions. In addition, long-term growth parameters

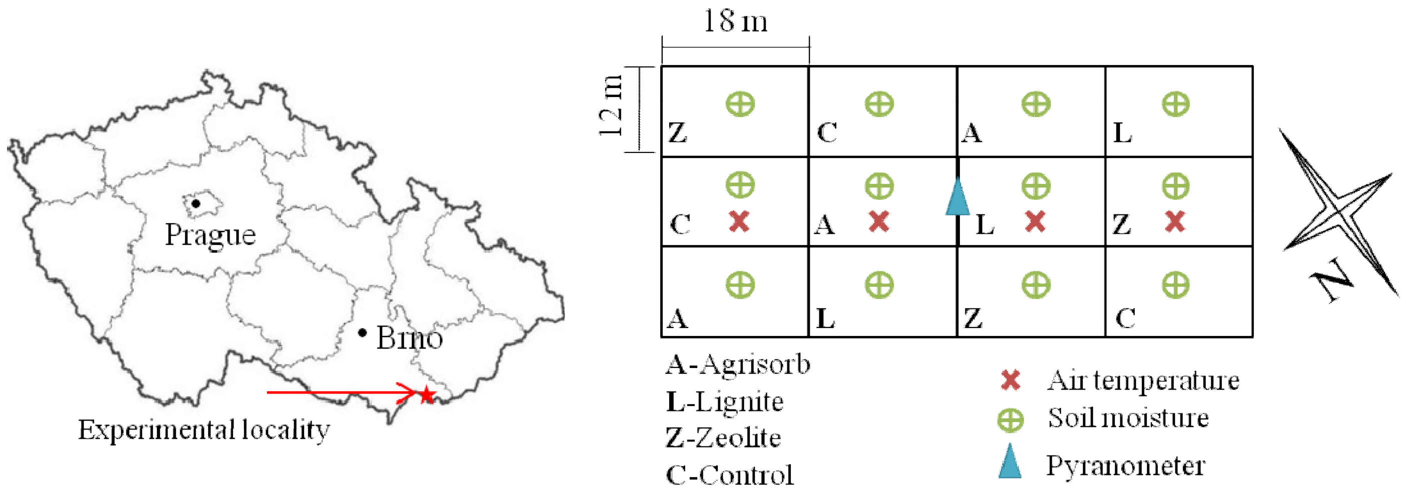

Figure 1. Map of the Czech Republic with experimental locality and design of experimental plots.

Table 1. Content of basic elements- phosphorus, potassium, magnesium, calcium, and ash. Values are represented in (mg·kg^{-1}), ash is expressed in (% of mass basis), n = 3.

Parameter	Value
P (mg·kg^{-1})	216 ± 14
K (mg·kg^{-1})	91 ± 7
Mg (mg·kg^{-1})	37 ± 6
Ca (mg·kg^{-1})	434 ± 28
Ash (%)	1.3 ± 0.2

Table 2. Soil particleð size fraction distribution. Fractions are expressed in (%), n = 3.

Soil horizon	Depth (cm)	Fraction (mm)								
		2.00–0.25	0.25–0.05	0.05–0.01	0.01–0.001	<0.001	<0.01	2.00–0.05	0.05–0.002	<0.002
Ap	5ï 10	54.5 ± 1	36.9 ± 1.4	2.5 ± 0.2	0.9 ± 0.1	4.9 ± 0.2	5.8 ± 0.5	91.5 ± 1.8	3.5 ± 0.2	4.8 ± 0.2
Ap	25ï 30	53.9 ± 1.1	36.7 ± 0.9	3.6 ± 0.3	0.8 ± 0.1	4.9 ± 0.3	5.7 ± 0.3	90.6 ± 1.7	4.3 ± 0.3	4.9 ± 0.3
AC	45ï 50	49.3 ± 1.2	40.0 ± 1.4	4.6 ± 0.4	0.6 ± 0.1	5.3 ± 0.3	5.9 ± 0.2	89.4 ± 2.1	5.2 ± 0.1	5.3 ± 0.2
AC	65ï 70	50.1 ± 0.8	40.7 ± 1.1	4.5 ± 0.4	0.1 ± 0.01	4.3 ± 0.4	4.5 ± 0.3	90.9 ± 2.2	4.4 ± 0.2	4.5 ± 0.3

such as height and diameter of the stem determined at the base and expressed in mm were determined.

Soil characteristics

Soil conditions of chosen locality are extreme and are summarized in Tables 1 to 5: content of basic elements (Table 1), regosoil (sthenic), size fractionï sand (Table 2), low water holding (retention) capacity (Table 3), high aeration (Table 4), and acidic soil reaction (Table 5). Classification of the soil as a sandy soil (group of light soils) is well evident from introduced parameters.

Supplementary soil substances

Three different supplementary soil substances were chosen: lignite, zeolite and hydroabsorbent *Agrisorb*. Natural unadjusted lignite

Table 3. Water and air characteristics of the soil.

Depth (cm)	KMK (%)	A (%)	MVK (%)	Θ (%)	Θz (mm)
5ï 10	34.99 ± 1.66	37.10 ± 6.88	9.82 ± 1.81	7.71 ± 3.91	25.75
25ï 30	23.24 ± 0.57	23.68 ± 0.76	9.90 ± 0.47	9.46 ± 0.55	

KMK (%), Maximum water capillary capacity; A (%), aeration; MVK (%), minimal soil air capacity; Ū (%), actual soil moisture; Ūz (mm), available (storage) soil moisture in depth of 30 cm; n = 3.

Table 4. Soil weight volume and porosity.

Depth (cm)	ρd (kg·m^{-3})	P (%)	Pk_{vol} (%)	Pk_{rel} (%)	Pn (%)
5ï 10	1,496 ± 44	43.2 ± 1.7	27.6 ± 3.9	61.5 ± 5.0	4.9 ± 0.7
25ï 30	1,720 ± 28	35.1 ± 1.0	16.2 ± 2.2	48.8 ± 5.1	5.0 ± 0.6

ȷ d (kg·m^3), Volume weight of dry soil; P (%), total porosity; Pk_{vol} (%), capillary pore space volume; Pk_{rel} (%), rate of capillary pore spaces; Pn (%), non-capillary pore space volume; n = 3.

Table 5. Soil reaction and content of soil organic matter of described soil horizons.

Horizon	Depth (cm)	pH_{H2O}	pH_{KCl}	Co_x (%)	OH (%)
Ap	5ï 10	6.1 ± 0.2	4.4 ± 0.2	0.6 ± 0.1	1.0 ± 0.1
Ap	25ï 30	5.8 ± 0.1	4.3 ± 0.2	0.3 ± 0.1	0.5 ± 0.1
AC	45ï 50	6.1 ± 0.2	4.9 ± 0.1	0.1 ± 0.03	0.2 ± 0.1
AC	65ï 70	6.0 ± 0.2	5.2 ± 0.2	0.1 ± 0.04	0.1 ± 0.04

pH_{H2O}, Active soil reaction; pH_{KCl}, exchange soil reaction; Co_x (%), content of oxidizable carbon; OH (%), content of soil organic matter; n = 3.

(Lignit Hodonín s.r.o,Hodonín, Czech Republic) represents ortholignite with the very low rate of carbonification. Lignite is brownish-black in colour with carbon content between 25 to 35% on mass basis and inherent moisture content approximately 66% on mass basis. The ash content of lignite is ranging between 6 to 19% on mass basis. The applied dosage was 500 gm^{ï2}. Further characteristics of lignite are introduced in Table 6. Zeolite (Bioclean, Repiste, Czech Republic) is a highly microporous natural supplementary soil substance of volcanic origin (tetragonal sodium aluminosilicate) containing approximately 70% of silicon dioxide on mass basis. Due to its microporosity, zeolite has ability to absorb high amount of water and ions with subsequent slow release. We used zeolite with a particle size fraction of 2.5 to 5 mm with an applied dosage was 300 gm^{ï2} in our experiments. Commercially available *Agrisorb* (Stockhausen GmbH&Co, Krefeld, Germany) represents synthetic organic polymer of the potassium polyacrylate with the extreme ability of water absorption with possibility of its subsequent release into soil. 1 g of the *Agrisorb* is able to absorb approximately 300 g of the water. Applied dosage was 200 gm^{ï2}. Supplementary soil substances were applied into the soil that was ploughed in the autumn, cultivated in the spring and subsequently aligned. The application depth was 0 to 100 mm.

Measurement of physiological parameters

For the determination of physiological state of experimental plants, the following parameters were determined: chlorophyll content, chlorophyll fluorescence, stomatal conductance, and sap flow. All chosen methods for measurement of above-mentioned physiological parameters were non-destructive. Measurement of the parameters was carried out in one hour intervals and was done by the following number of sensors: three instruments for measuring chlorophyll content, three for measuring chlorophyll fluorescence measurements and three for stomatal conductance. Measurement of chlorophyll fluorescence, chlorophyll content and measuring of stomatal conductance takes place always on the same leaf, each measurement was done in triplicates (n = 3). A representative leaf as the third leaf from the terminal shoot was identified and was used for all measurements during the entire growing season. The age of leaves was about four months. Chlorophyll contents were determined with a chlorophyll content meter CCM-200 (Opti-Sciences, Hudson, NH, USA). Optical absorbance was measured at two wavelengths (653 nm and 931 nm) to measure chlorophyll content considering leaf thickness. Measurement area was 0.71 mm^2. A silicon photodiode with integral amplifier for absorption measurement was used as a detector with mode compensating temperature. Chlorophyll content in leaves was determined in relative values.

Non-destructive chlorophyll fluorescence measurements were carried out with an Opti-Sciences OS-30 chlorophyll fluorometer (Opti-Sciences, Hudson, NH, USA), as a measure of photosynthetic efficiency. The pulse modulating high resolution sampling mode for Kautsky induction curve recording was selected as detection method. The photodiode of chlorophyll fluorometer with a 700 to 750 nm band pass filter served as a detector. "Clips" were laid on the leaves of plants to darken leaves, which lasted for 15 minutes and then fluorescence was measured. The excitation source of chlorophyll fluorometer was solid state 660 nm source modulation beam adjustable 0.2 to 1.0 µE saturation/actinic intensity adjustable from 100 to 3,000 µE depending on the test mode. For the determination itself, the quantum yield for PSII was determined using ration variable fluorescence (Fv) and maximal fluorescence

Table 6. Basic characteristic of lignite, n = 3.

Characteristic (%)	Sample	Water-free sample	Sample with inflammable modification
Free water	41.30	-	-
Bound water	4.46	-	-
Total water	45.76	-	-
Ashes	16.73	30.85	-
Flammable material	37.51	69.15	100
Volatile matter	21.65	39.91	57.72
Non-volatile matter	15.86	29.24	42.28
Hydrogen	2.10	3.88	5.61
Carbon	24.28	44.77	64.74
Nitrogen	0.37	0.68	0.98
Oxygen	10.28	18.95	27.41
Volatile sulphur	0.48	0.87	1.26
Sulphated ash	0.37	0.69	-
Total sulphur	0.85	1.56	-

(Fm). Measurements of stomatal conductance were carried out using non-destructive method by using a Porometer AP4 (Delta-T, London, United Kingdom). The AP4 measurements were based on diffusion conductance by comparing the precise rate of humidification within a small cuvette placed on calibration plate. The plate had six diffusion conductance settings whose values were accurately determined by element analysis. Values of stomatal conductance of leaves expressed in values of [$mmol \cdot m^{-2} \cdot s^{-1}$] were determined. Proportionally, same place of the leaves of the same onthogenetical stage of development was used for the measurement of chlorophyll content, chlorophyll fluorescence, and stomatal conductance. Measurements were carried out in triplicates for the statistical evaluation. Sap flow was monitored using Dynagage sap flow sensors (Dynamax, Houston, TX, USA) based on the method of heat balance (Heat Balance Method) with SGA5-ws sensors. These energy balance sensors measure the amount of heat carried by the sap, which is converted into real-time sap flow in grams per hour [gh^{-1}]. Unlike other methods, this method requires no calibration because sap flow is directly determined by the energy balance and rates of heat convection by the sap flow. Monitored physiological parameters were determined at the same time to compare the obtained results. In addition, all results were statistically related to the microclimatic characteristics of field localities.

Measurement of morphological parameters

Plant height increase was determined using measuring scale (IRA, Mumbai, India) at the height of terminal bud with a measurement accuracy 1 mm. Radial increase was monitored using an EDC15M digital slide gauge (Omicron, Trebic, Czech Republic) at the height of root collar with an accuracy to tenth of a mm. All measurements were carried out at the same time (November 15th, 2009 and November 15th, 2010).

Microclimatic measurement

Solar radiation intensity was determined using a pyranometer SG 002 (Fiedler-Mágr, Ceske Budejovice, Czech Republic) with a measurement range from 0 to 1,200 $W \cdot m^{-1}$. Intensity of solar radiation intensities were measured at strictly defined half-hour intervals. For the determination of air temperature and relative humidity, HOBO sensors (Onset Computer Corporation, Bourne, MA, USA) were used. Temperature was determined in the height of one meter per 15 minutes long time intervals.

These parameters were determined for each of experimental plots. VIRRIB sensors (Amet, Velke Bilovice, Czech Republic) were used for the soil moisture measurement. Obtained data were transmitted to the data logger with a digital processor enabling measurement of current (frequency 4 MHz). The sensors work on the time domain transmission (TDT) principle. Measurement output consists at a frequency at 4 MHz of Current loop (0 to 5 mA, 1 mA = 10 volume percentage), and is applicable up to the 50% soil moisture. Soil moisture was measured at depths of 0.15, 0.30 and 0.45 m through a soil profile.

Mathematical and statistical analysis

Linear regressions were used to estimate parameter interdependence. Most parameters manifested strictly linear character; therefore, method of least squares of the first order was sufficient for this purpose. The inaccuracy of the estimated linear regression was calculated as the sum of squared deviations from a linear dependence, which was expressed by the equation of the linear regressions. For this purpose, the MATLAB (MathWorks Inc., Natick, USA) Statistic at Toolbox and statistical functions in MS Excel (Microsoft, Redmond, USA) were used. To statistically process 120 hour long continuous monitoring of physiological parameters, 1D interpolation of the measured parameters and the calculation of the area under the curve were used and calculated by MATLAB Signal Processing Toolbox. Statistical significances of the differences between parameters of interest were determined using STATISTICA.CZ (Czech Republic). Differences with $p < 0.05$ were considered significant and were determined by using of one way analysis of variance (ANOVA) test (particularly Scheffe test), which was applied for means comparison.

RESULTS AND DISCUSSION

Chlorophyll content and fluorescence, sap flow and stomatal conductivity

Changes in chlorophyll content in plants are closely associated to their actual physiological state and given

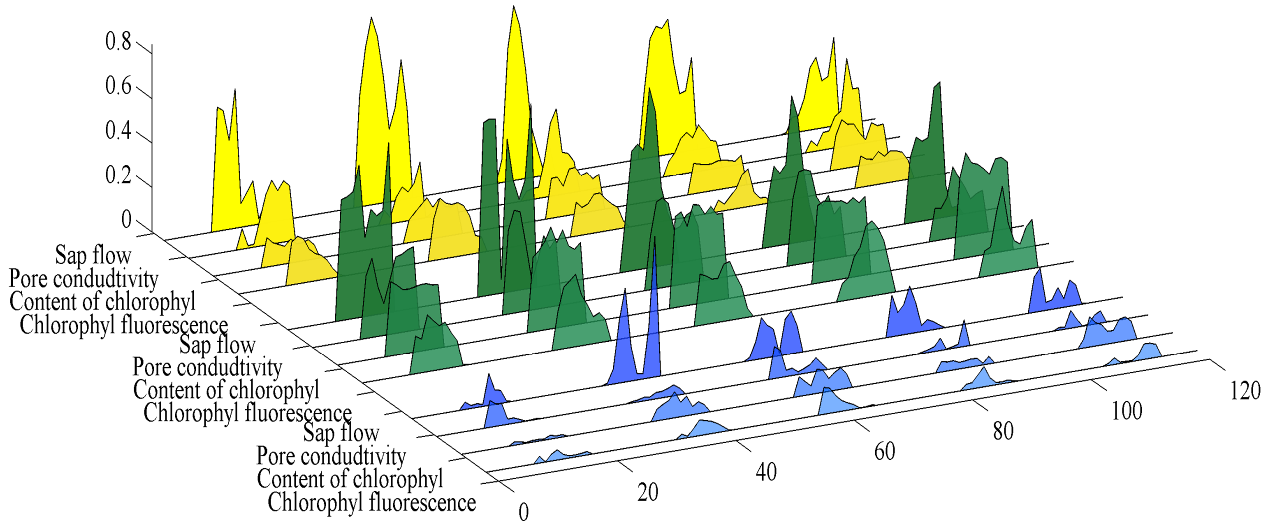

Figure 2. Normalized values of studied physiological parameters as sap flow (relative standard deviations (RSD) 2.9%, number of measurements (n) = 3), stomatal conductivity (RSD = 2.4%, n = 3), chlorophyll content (relative standard deviations (RSD) = 1.8%, n = 3) and chlorophyll fluorescence (expressed as ratio of maximum chlorophyll fluorescence (Fm) and variable chlorophyll fluorescence (Fv) values. RSD = 3.4%, n = 3) measured in *Acer campestre* L. (field maple) planted in the presence of *Agrisorb*, lignite and zeolite. All determined values are relative to the control.

conditions, such as intensity and direction of irradiation,water availability or presence of mineral nutrients (Freiberger et al., 2010; Jin et al., 2011; Ma et al., 2011). Photosystem II can be negatively affected by various types of stresses including pollutants as metal ions (Adam et al., 2010; Diopan et al., 2010; Klejdus et al., 2004; Petrlova et al., 2006; Potesil et al., 2005; Supalkova et al., 2007a; Zitka et al., 2011a; Zitka et al., 2011b), anthropogenic activity and/or low sources of water, which belongs to the group of the most important factors inhibiting photosynthesis (Kauser et al., 2006). It was demonstrated that decreased photosynthesis under water stress is associated with perturbations of biochemical processes. Water stresses easily affect photosystem II and can damage the oxygen-evolving complexes. The rate between F_V and F_M expressing maximal quantum yield of photosystem II is usually used as general indicator of the decrease of function or damage of reactive centres of phytosystem II. Measurement of chlorophyll fluorescence provides information about activity of photosynthetic apparatus under stress conditions. Methods based on chlorophyll fluorescence are increasingly used, especially due to advantages of these techniques as estimation of intensity and time duration of stress conditions in time (Lichtenthaler et al., 2005; Longenberger et al., 2009; O'Neill et al., 2006).

Photosynthesis and sap flow are in very close relation. Sap transported represents medium containing not only products of photosynthesis, such as sugars, but also amino-acids and ribonucleic acids, which are important for regulation of physiological processes not only in autotrophic but also in heterotrophic plant tissues. Sap flow measurement is closely connected with total water consumption by plants and provides data about transport of nutrients (Conejero et al., 2007; Ortuno et al., 2005). Sap flow provides information about actual water relations in plants. Modern instruments designed for determination of sap flow are non-invasive and based on the energy balance derived from the constant source of heat recording sap flow in stems (Asbjornsen et al., 2011; Dalsgaard et al., 2011; Kucerova et al., 2010). Under damaged water balance plants close the stomata due to reduction of transpiration with the aim to decrease water loss. Closed stomata are associated with depletion of carbon dioxide uptake, which results in deceleration of photosynthesis. Closing of stomata can be measured by porometers (Blonquist et al., 2009). Determination of actual stomata state is needed for studying of photosynthesis rate (Moureaux et al., 2008).

Figure 2 introduces and graphically expresses physiological parameters measured during five-day long field experiment in the experimental plots for the control and for lignite, zeolite and *Agrisorb* plots. Values are related to the control, in which the control variant was considered as 0. All data are present as mean values. The highest chlorophyll content was in plants cultivated on the soil supplemented by lignite. The highest value was determined in the last experimental day (5th day). Chlorophyll contents were 13 relative units in control, 13.7 relative units in plants cultivated on the soil supplemented with *Agrisorb*, 17.8 relative units in plants cultivated on the soil supplemented with lignite, and 14.9 relative units in plants cultivated on the soil supplemented

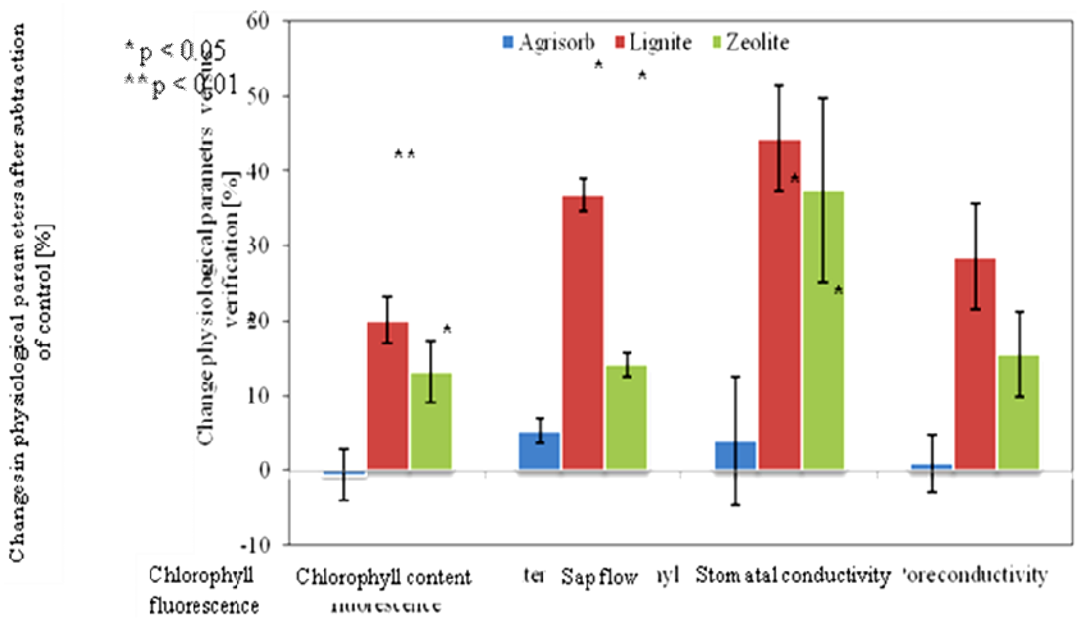

Figure 3. Comparison of physiological parameters (sap flow, stomatal conductivity, chlorophyll content, and chlorophyll fluorescence) measured in *Acer campestre* L. (field maple) planted under influence supplementation by supplementary soil substances (*Agrisorb*, lignite and zeolite). Values are related to values of control variant without supplementary soil substances.

with zeolite (Figure 2). Chlorophyll contents were higher for app. 5% in plants cultivated on the experimental plots supplemented by hydroabsorbent *Agrisorb* compared to control plants. Similarly, compared to control, chlorophyll contents were higher for 37 and for 14% in plants cultivated on the soil supplemented with lignite and zeolite, respectively. Differences in chlorophyll content were closely connected not only with changes in the activity of enzymes connected with chlorophyll biosynthesis, but also with chlorophyll degradation.

Values of maximal quantum yield of photosystem II during five day long experiment are shown in Figure 2. In the case of application of zeolite, values of the rate of maximal and variable fluorescence (maximal quantum yield of photochemistry of photosystem II) were 0.54. In the experimental plots supplemented with *Agrisorb*, the same value of the rate of maximal and variable fluorescence was determined compared to control plants. The highest quantum yield of photosystem II (0.65) was observed in plants cultivated on the soil supplemented with lignite, which means 20% higher value in comparison with control plants. Supplementation by zeolite caused 13% enhancement compared to control variant. Based on the results obtained it can be concluded that the application of lignite to the soil markedly increased maximal quantum yield of photosystem II of plants compared to control. The activity of photosystem II was higher also in the experimental variant cultivated in the presence of soil supplemented by zeolite. However, *Agrisorb* treated plots were similar to control. We were also interested in the changes of stomatal conductivity in leaves of plants cultivated on the soils with various supplementary soil substances. Values of stomatal conductivity were 500 mmol·m^{-2}·s^{-1} in control plants. Application of *Agrisorb* led to only minimal changes in values of stomatal conductivity. The conductivities were 505 mmol·m^{-2}·s^{-1}, which represents 1% higher value compared to control. The highest value as 643 mmol·m^{-2}·s^{-1} were determined in the plants cultivated in the soil supplemented with lignite (Figure 2). Compared to control plants, application of zeolite increased stomatal conductivity values to 578 mmol·m^{-2}·s^{-1}. The enhanced stomatal conductivity can be clearly associated with the increasing water accumulation.

Sap flow of control plants was determined as 5.6 g·h^{-1}. In the case of *Agrisorb* application, the detected value was 5.8 g·h^{-1}, which was 3% enhancement compared to control plants. The supplementation of lignite led to increase this value to 8.1 g·h^{-1}, which was 44% higher in comparison with the control plants. For zeolite, a value of 7.7 g·h^{-1} (36% higher compared to control) was detected (Figure 2). Based on the results it can be concluded that application of lignite and zeolite caused very similar enhancement of sap flow in the experimental plants during the experiment. In the last day of the experiment, plants cultivated in the soil supplemented with zeolite had higher sap flow compared to other experimental variants. Application of *Agrisorb* did not cause any changes compared to control plants.

For the better clarity and comparability of the obtained data, all physiological parameters were normalized.

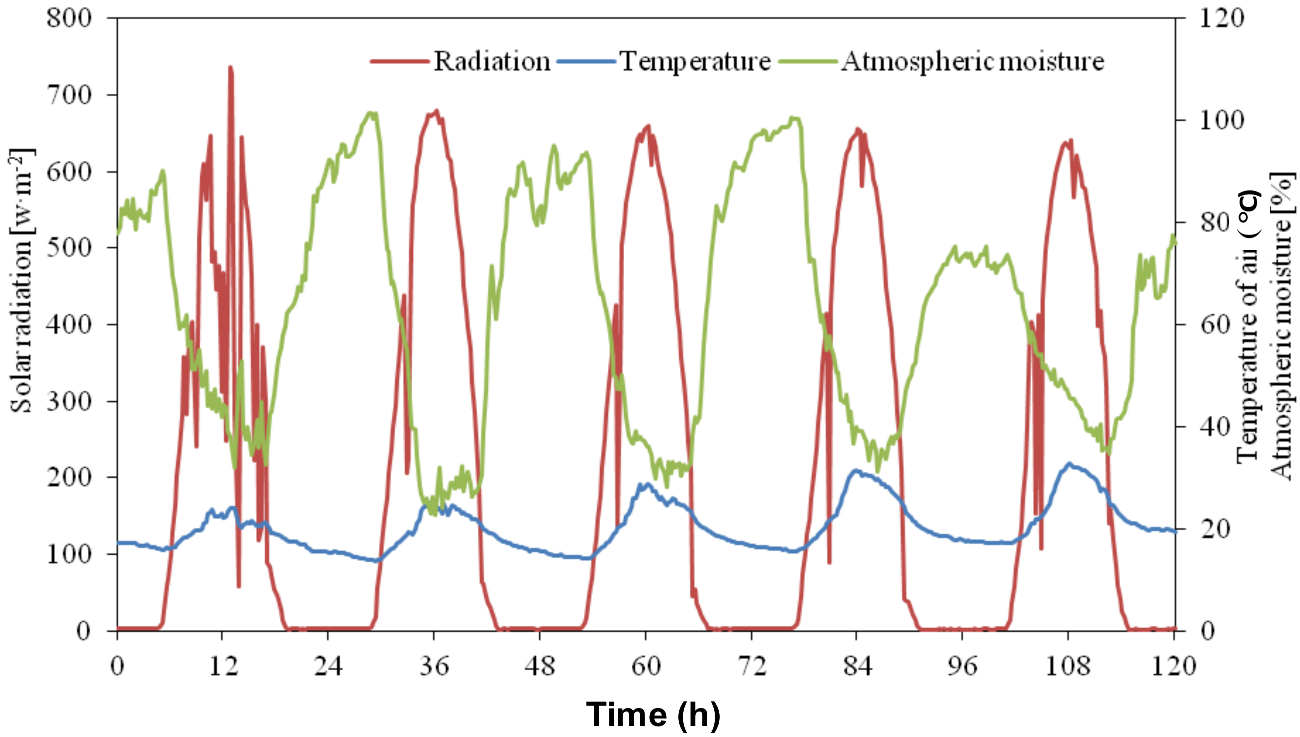

Figure 4. Changes in solar radiation, air temperature, and air relative humidity at the time of measurement of physiological parameters (sap flow, stomatal conductivity, chlorophyll content, and chlorophyll fluorescence). RSD = 2% (solar radiation), 0.5% (temperature), 2% (air atmospheric moisture).

Table 7. Values of correlation coefficients between physiological (sap flow, stomatal conductivity, chlorophyll content and chlorophyll fluorescence) and microclimatic parameters (solar radiation, air temperature and air atmospheric moisture).

Data correlation	Chlorophyll fluorescence	Content of chlorophyll	Sap flow	Stomatal conductance
Radiation	0.911	0.864	0.926	0.906
Temperature	0.946	0.899	0.944	0.939
Relative humidity	ī 0.843	ī 0.852	ī 0.831	ī 0.861

Figure 3 shows values of parameters normalized to the relative values after integration of the areas under curves shown in Figure 2. Control variants for individual physiological parameters were expressed by the value of 0%. In the case of applying lignite, chlorophyll fluorescence, sap flow and stomatal conductivity were significantly higher compared to control at $p < 0.05$. Chlorophyll content enhanced significantly after application of lignite at $p < 0.01$. All studied parameters were also significantly enhanced in plants cultivated in the presence of zeolite. The results obtained after application of *Agrisorb* did not significantly differ from the control ones (Figure 3).

Correlation of physiological parameters with microclimatic conditions

All monitored physiological parameters were related to the data about microclimatic conditions with the focus to determine connection between climate (weather) and values of physiological parameters (Figure 4). Results demonstrating rates of dependence of physiological parameters on monitored microclimatic parameters are summarized in Table 7. Analysis of data showed high correlation between chlorophyll fluorescence, sap flow and stomatal conductivity and course of solar radiation and air temperatures. Chlorophyll contents were in correlation with these parameters too, but they are not directly dependent on microclimate.

Based on the calculated correlation coefficients it can therefore be concluded that the chlorophyll fluorescence, sap flow and stomatal conductance decrease due to lower temperatures and less favourable light conditions. All of these physiological parameters were negatively correlated with relative humidity. On the other hand, we expected t hat the monitored parameters would be

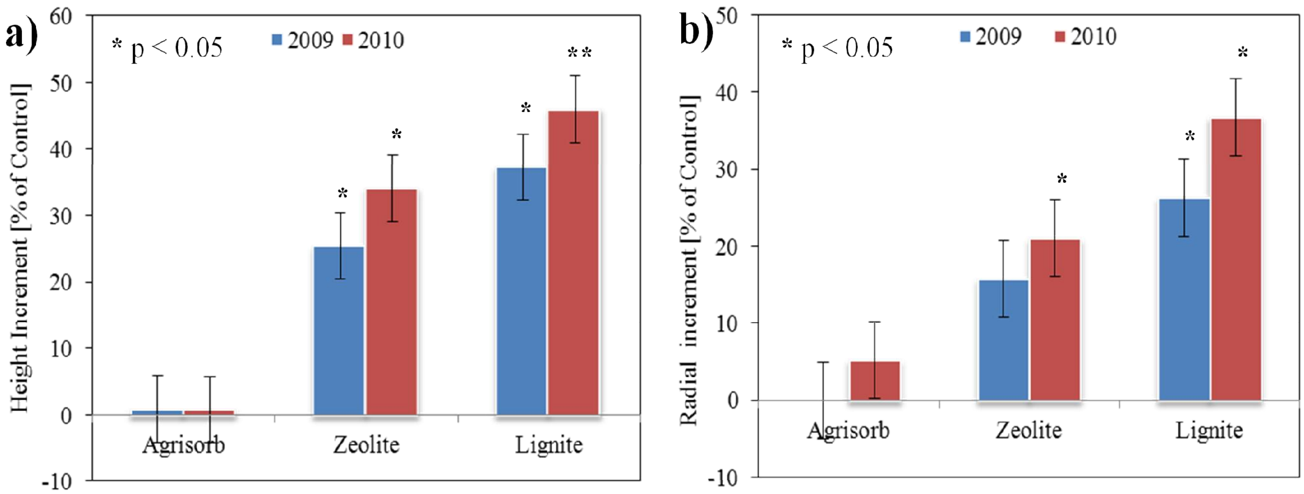

Figure 5. Average height (**a**) and radial (**b**) increment expressed in [%] measured in experimental plants planted in the presence of *Agrisorb*, lignite and zeolite. Results were obtained in 2009 and 2010, RSD = 0.5%, n = 3.

positively correlated with this variable. This fact would be applied if it would not change other microclimatic parameters. In the case of a field experiment, however, solar radiation and temperature were more significant impact than relative humidity on the physiological characteristics. Due to the fact that the relative humidity increased, correlation coefficients were negative.

Height and radial increment

During two years, selected morphological parameters as height increment and radial increment at the site of root collar were monitored. Height increment of control plants was related to 0%, which means that their increase in the height was considered as 0% and was used as a base for evaluation of other parameters Average values for control plants were as follows: 0.118 m (2009) and 0.126 m (2010). These parameters can be used as markers of long-term and sustainable improvement of soil characteristics due to the presence of supplementary soil substances. The highest height increment was detected in experimental plants cultivated in the soil supplemented with lignite (Figure 5). Their height increment was 37% (2009) and 46% (2010) compared to control plants. The increase was significant at $p < 0.05$ in 2009 and at $p < 0.01$ in 2010. In the case of zeolite, the height increment was 25% (2009) and 34% (2010) compared to control plants. Both differences were found to be significant at $p < 0.05$. The lowest height increment as 1% for both years (2009 and 2010) was determined in the case of aplication of hydroabsorbent *Agrisorb*, compared to the control plants (Figure 5).

Values of radial increment detected in the site of root collar were related to control variant and recalculated to the relative percentages (Figure 5). Average value of control plants was 0% with the radial increment 0.95 mm for both years (2009 and 2010). The highest average radial increment was determined in plants cultivated on the soil supplemented with lignite. The value determined in 2009 was higher for 26% and in 2010 for 37% in comparison with control plants, which was followed by plants cultivated on the soil supplemented with zeolite (16% increment in 2009 and 21% for 2010). Radial increments plants cultivated on the soil supplemented with *Agrisorb* were comparable to control plants. In the year 2009, they were the same as the radial increments of control plants, in year 2010 the increment was only 5% compared to control plants.

Soil moisture

Soil moisture was monitored in all experimental plots. Figure 6 demonstrates courses of curves determining soil moisture in all four experimental plots during experiment through soil profile in the depth from 0.05 to 0.25 m (Figure 6a) and in the depths of 0.15 m (Figure 6b), 0.30 m (Figure 6c), and 0.45 m (Figure 6d). This parameter as the main factor determining physiological and growth state of plants is a marker of the positive effect of supplementary soil substances. Through the increasing values of soil moisture, the positive effect of supplementary soil substances on plants may be explained. The highest value of soil moisture was detected for lignite application compared to control plants. In addition, physiological parameters measured in the plants cultivated in the soil supplemented by lignite were the highest. The second highest value of soil moisture was determined in the case of supplementation

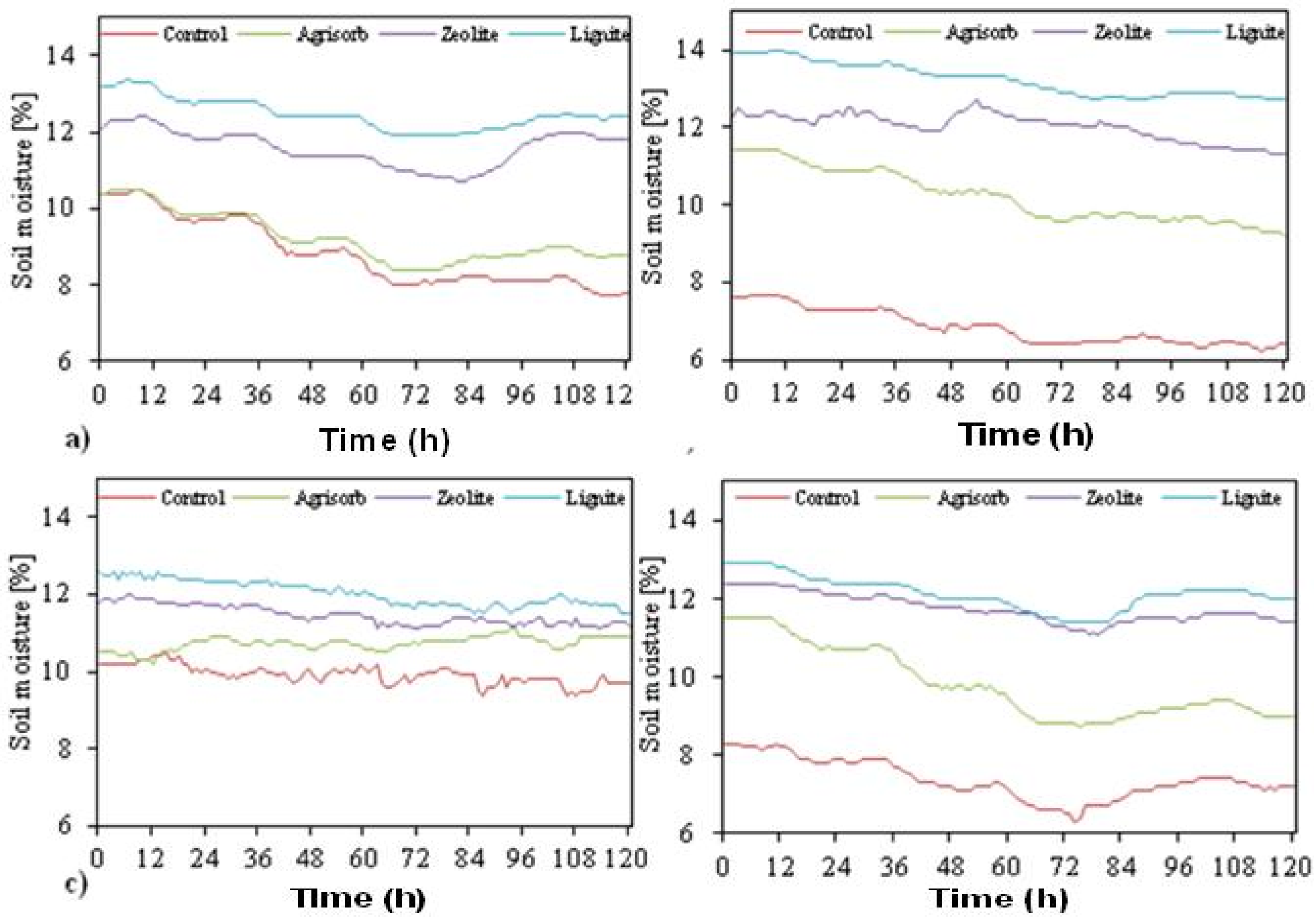

Figure 6. Values of soil moisture detected using VIRRIB sensors during 120 hours in the depth of 0.05 to 0.25 (a), 0.15 (b), 0.30 (c) and 0.45 m (d). Results are expressed for application of *Agrisorb*, lignite and zeolite, and control without application of soil supplementary substance. RSD = 2%.

of soil with zeolite. This finding corresponded to values of physiological parameters, especially maximal quantum of soil with zeolite. This finding corresponded to values of physiological parameters, especially maximal quantum yield of photochemistry of photosystem II. The third highest value was observed in the case of *Agrisorb*, the lowest values of soil moisture were determined in control variant without supplementary soil substances supplementation. In conclusion, all these findings correspond to the monitored physiological, but also growth parameters of experimental plants of *A. campestre* L.

To evaluate the influence of soil moisture on the physiological and morphological parameters, correlograms were used and are shown in Figure 7. We calculated the average soil moisture at 15 cm for all three types of soil materials. These values are set proportionally to the average moisture control measurements. In the same way we also obtained the proportional value of physiological and morphological parameters. The correlation between the proportional values of soil moisture and physiological parameters shows a significant linear relationship (Figure 7a). We expressed the equations of the lines and confidence coefficient as the square of deviations from the linear progression. The obtained figure is divided into three segments according to the type of supplementary soil substance. It is evident that lignite has the greatest influence on the physiological parameters and *Agrisorb* the smallest one. Increasing humidity increases all physiological parameters, however the greatest influence of soil moisture was observed on the sap flow, while the smallest one on chlorophyll fluorescence. Deviations from the linear regression are above 90% except chlorophyll content. It is evident that an exponential increase in the chlorophyll content begins to show at higher moisture content.

Mutual correlations

As mentioned above in the case of physiological parameters, we investigated the degree of correlation for

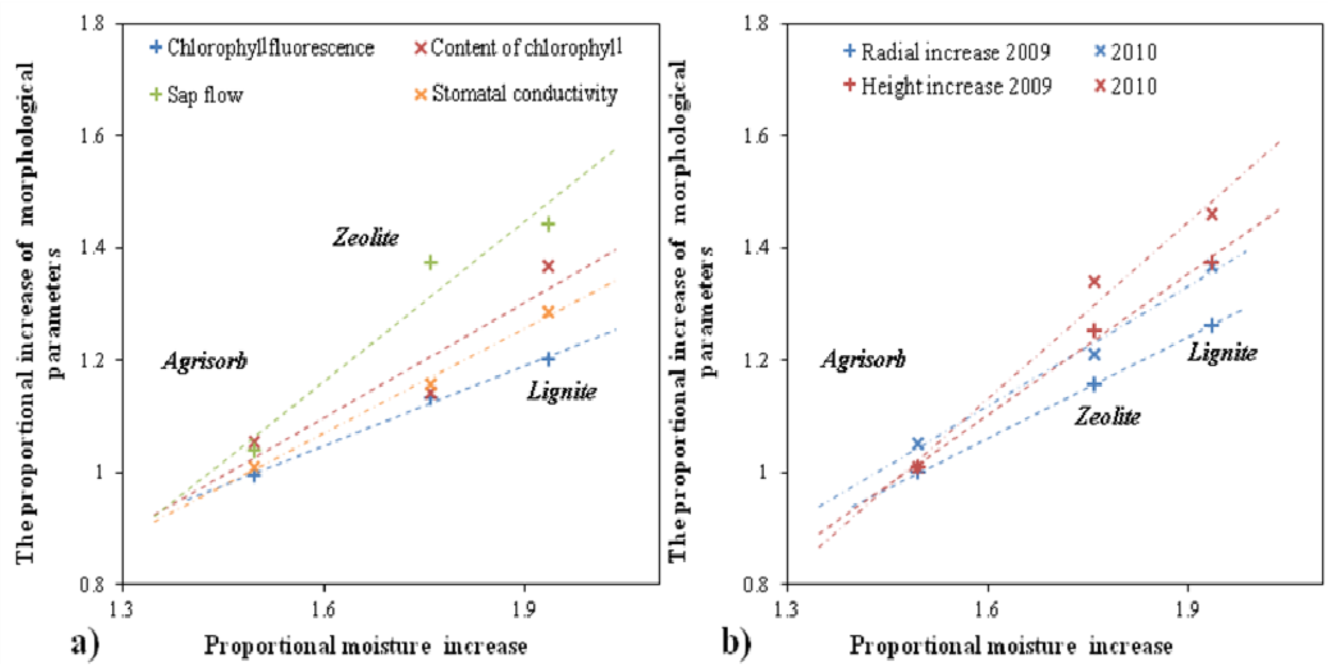

Figure 7. Correlograms of influence of soil moisture on (**a**) physiological (sap flow, pore conductivity, chlorophyll content and chlorophyll fluorescence) and (**b**) morphological parameters (radial increase, height increase).

morphological parameters. First, we found a linear dependence of proportional increase in the value of morphological parameters of height and thickness of maple, depending on soil moisture. Due to the fact that the highest moisture was found in lignite, this supplementary soil substance causes the highest increment in the growth of experimental plants (Figure 7b). Strictly linear dependence confirms again confidence coefficient value determined above 97% in terms of linear functions to increase the thickness in 2009 (R 2009) and in 2010 (R 2010), as growth in the form of plant height measured in both years (H 2009, H 2010). Slope of the height increase of plants is significantly higher than the growth in thickness depending on the humidity, which regression equation also shows. Considering the fact that the slope of the dependence of the thickness is equal to 0.601 for 2009 and 0.712 in 2010, in the corresponding years, slope of the dependence of increase in height was 0.841 for 2009 and 1.052 for 2010.

We also calculated correlations of physiological parameters in relation to growth parameters. The proportional nature of all the values was necessary due to the vastly different values of physiological parameters. Then we therefore designed four correlograms (Figure 8), two for the increase in thickness between 2009 and 2010 and two for the increase in height during these years, depending on all four physiological parameters expressed as the proportional increase in the control. There is a significant linear dependence of all correlated values, with minor inaccuracies in the flow of sap and chlorophyll content. In the first mentioned parameter, confidence coefficient varied from 87 to 99%, in the second case from 75 to 93%. We can therefore assume the nonlinear process with longer time period and therefore with the growth of plants. The chlorophyll content and sap flow show the smallest increase of phy-siological parameters depending on the growth of the plant. How physiological characteristics correlated between each other was also studied. The calculation included the values from all tested soil supplementary substances (*Agrisorb*, zeolite, lignite, control). Based on the resulting values of correlation coefficients we can assume that the observed physiological parameters are mutually dependent and strongly interact (Table 8).

In our case, application of zeolite led to improvement of growth and physiological parameters of *A. campestre* L. Improvement of nitrogen availability by the use of zeolite has been demonstrated (Omar et al., 2010). Connection between carbon, its retaining in soil systems and accumulation in biomass is discussed in Wang et al. (2010). The authors explained better growth and quality of fruits in association with carbon accumulation in plants. The best results in physiological and growth parameters of experimental plants were obtained in the case of lignite usage in our study. It is obvious that lignite containing high carbon content may contribute to better accumulation of water in plants. Effect of *Agrisorb* on experimental plants was comparable to effect of unsupplied soil. Some authors describe usage of compounds which are able to bound not only water,

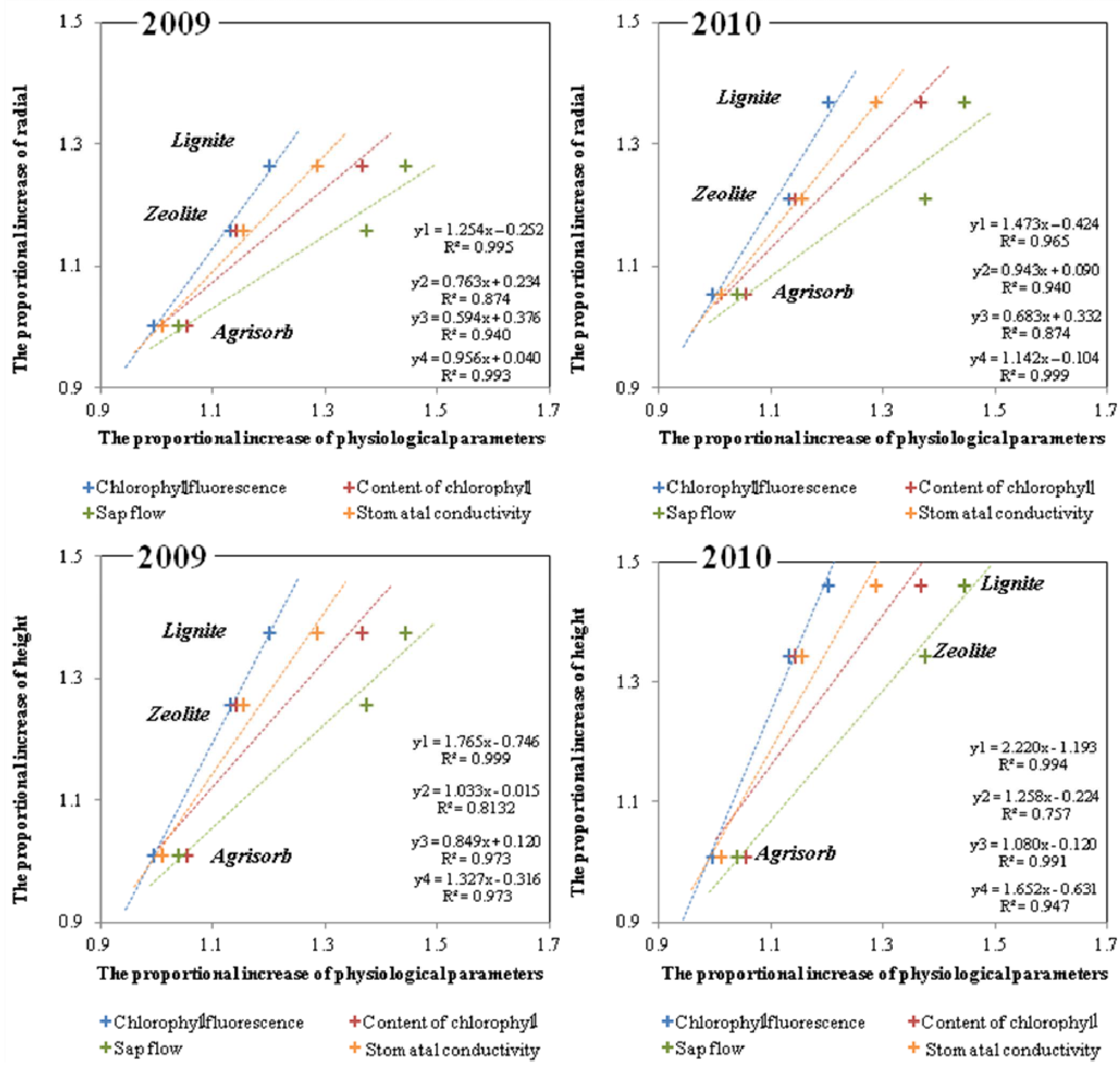

Figure 8. Correlograms showing the relation between physiological (sap flow, pore conductivity, chlorophyll content and chlorophyll fluorescence) and growth (radial increase, height increase) parameters of experimental plants.

but also salt in soil (Dorraji et al., 2010). Positive effects on growth of plants are connected with reduction of soil salinity. On the other hand, in the case of hydroabsorbent *Agrisorb*, water may be very strongly bound in hydrogel under its low availability to plant. This theory can successfully explain no positive effect of supplementation of soil by *Agrisorb*.

Conclusion

In conclusion, the importance of this work consists not only in the connection between usage of soil supplementary substances and growth and physiological parameters, but also in usage of sensors for monitoring of actual conditions, which are connected for example with actual climatic conditions or soil moisture. The application of these techniques to assess the ability of various soil supplementary substances to improve moisture availability that would affect plant physiology is the contribution of this report. It is well evident that non-destructive sensors of long-term monitoring are useful in the study of growth and physiological processes of

Table 8. Values of correlation coefficients between physiological parameters.

Parameter	Chlorophyll content	Sap flow	Pore conductivity
Chlorophyll fluorescence	0.934	0.984	0.991
Chlorophyll content	-	0.883	0.973
Sap flow	-	-	0.961

plants. Supplementation of soil by three different types of supplementary soil substances (lignite, zeolite and hydroabsorbent *Agrisorb*) led to different results. Plants cultivated in soil supplemented by lignite and zeolite demonstrate better values of physiological and growth parameters. On the other hand, application of hydroabsorbent *Agrisorb* was comparable to cultivated in experimental plots without supplementary soil substances. Measurement of microclimatic parameters as solar radiation and air temperature highly correlated with physiological parameters as chlorophyll fluorescence, sap flow and pore conductivity. Values of soil moisture highly correlated with physiological and growth parameters of experimental plants. In the view of morphological characteristics, application of lignite and zeolite led to the significant enhancement of both height and radial increments. On the contrary, hydroabsorbent *Agrisorb* demonstrated only minimal changes in all morphological parameters in comparison with control plants. Obtained results show the possibility and bene-ficial effects of application of supplementary soil substances lignite and zeolite in drought degraded soils owing to global climatic changes.

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