

Comparative analyses of gas exchange characteristics and chlorophyll fluorescence of three dominant tree species during the vegetation season in hemi-boreal zone, Lithuania

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Abstract

Current climate scenarios predict rising air temperature along with increasing frequency and intensity of summer drought in the Central and Eastern Europe. Severe drought episodes affect physiological processes in trees such as transpiration, photosynthesis and carbon allocation. Understanding gas exchange between plants and the atmosphere is important in woody plant research. The aim of this study was to evaluate differences in gas exchange characteristics and chlorophyll fluorescence of tree species prevailing in Lithuania (Scots pine, Norway spruce and Silver birch) and their physiological response to water stress. The study was conducted in Aukštaitija integrated monitoring station, Lithuania. Gas exchange parameters and chlorophyll fluorescence were measured during the vegetation season of 2016. Meteorological parameters were obtained from the monitoring station. Four weather periods with different meteorological conditions were identified. Under moderate drought conditions all investigated tree species demonstrated reduced photosynthetic rates, lower stomatal conduction transpiration rates, water use efficiency and instantaneous carboxylation efficiency. During moderate drought, intercellular CO₂ concentration of Norway spruce was higher and this species demonstrated the highest decrease in instantaneous carboxylation efficiency. No significant changes of maximal chlorophyll fluorescence (Fv/Fm) among species were detected during different weather periods except Silver birch. The investigated tree species reacted differently to weather conditions. The Scots pine demonstrated the highest tolerance to different weather conditions. The study confirmed the sensitivity of Norway spruce to drought conditions. The Silver birch was the least sensitive to temperature and humidity conditions variation.

Key words: Climate change, Coniferous trees, Deciduous trees, Photosynthesis

1. Introduction

The impact of climate change on the forest ecosystems has been observed in many sites, which affects forest structure, functions, biodiversity, distribution, growth and productivity (Allen *et al.*, 2010; Eilmann and Rigling, 2012; Lei *et al.*, 2016; Kitaoka *et al.*, 2016; Augustaitis *et al.*, 2018).

Current climate scenarios predict rising air temperature along with increasing frequency and intensity of summer droughts in Central and Eastern Europe in the upcoming decades (IPCC, 2014). Recently forests have already globally and regionally suffered from drought and heat events (Walther, 2003; Serengil *et al.*, 2011; Eilmann and Rigling, 2012; Kozyr, 2014). Boreal forests probably will be severely affected by climate change (Ashraf *et al.*, 2015).

Tree species have limited possibility to adapt to changing

climatic conditions due to rapid climate change and the long life-span of trees (Eilmann and Rigling, 2012). The investigation of the physiological mechanisms through which dry and hot climatic conditions cause tree productivity decline and mortality is becoming more important (Allen *et al.*, 2010; Levesque *et al.*, 2013; Gao *et al.*, 2017). Tree species have developed number of adaptive mechanisms including the photochemical and biochemical systems to cope with drought stress. Adaptation to drought conditions includes both phenotypic and genotypic changes (Yordanov *et al.*, 2000). Tree species can maintain water potential, to adapt leaves, stems and roots seeking to restrict water loss (Kozłowski and Pallardy, 2002). Understanding the impact of future climate change on forest species is important for forest managers to generate adaptation and mitigation strategies (Scherer-Lorenzen *et al.*, 2005).

Terrestrial plants assimilate carbon dioxide (CO₂) during the process of photosynthesis and transpire water (H₂O) from the leaf stomata. Both processes are regulated by environmental factors and plant physiological processes. Plants save water through a reduction of stomatal conductance, which leads to decreased carbon uptake (McDowell *et al.*, 2008; Will *et al.*, 2013). Soil

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water deficiency can reduce transpiration (Bréda *et al.*, 1993; Clenciala *et al.*, 1998; Irvine *et al.*, 1998), and it is important limiting factor to plant photosynthesis in poor dry sandy soils.

Water Use Efficiency (WUE) is an important measure that evaluates the trade-off between photosynthetic carbon assimilation and transpiration at the leaf level (Medrano *et al.*, 2002; Lawlor and Tezara, 2009). WUE can be used to study forest ecosystem functioning in connection with cycles of water, energy, and carbon (Keenan *et al.*, 2013).

Severe drought episodes affect physiological processes in trees such as transpiration, photosynthesis and carbon allocation. This may lead to reduced primary productivity and even forest stand decline (Allen *et al.*, 2010; Matussek *et al.*, 2012).

The sensitivity of trees to climate change differs among species and regions (Zhang *et al.*, 2014; Lei *et al.*, 2016). Therefore, knowledge of species and region specific responses to climate is needed. In Lithuania, a hemi-boreal European region, Scots pine

(*Pinus sylvestris* L.), Norway spruce (*Picea abies* Karst.) and Silver birch (*Betula pendula* L.) are dominant tree species.

Scots pine is a light demanding pioneer species occurring in a wide range of habitats and thus is widely distributed over the world and is known as being tolerant to moderate drought (Matias and Jump, 2012; Lebourgeois *et al.*, 2012; Eilmann and Rigling, 2012). Norway spruce is an intermediately shade tolerant species widely spread in Europe and is sensitive to drought episodes (Lebourgeois *et al.*, 2010; Levesque *et al.*, 2013; Neuner *et al.*, 2015).

Silver birch is an early successional pioneer species widespread in the temperate and boreal forests of Europe (Hynynen *et al.*, 2010). Silver birch trees are fast growing and exceptionally tolerant to spring frost, low air temperatures and nutrient deficiency, growing in both wet and dry sites, although their ecological distribution is limited by shade intolerance and short life span (Ellenberg, 2009). In Northern and Eastern Europe, Silver

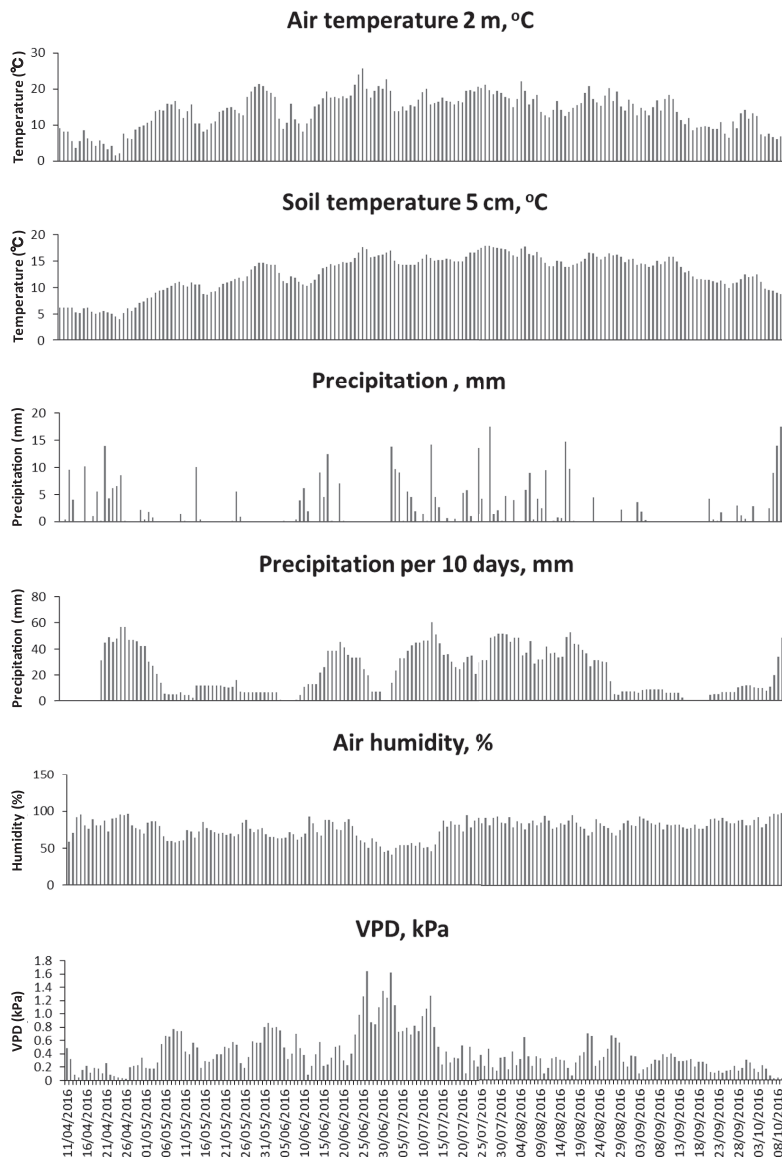


Fig. 1. Daily meteorological parameters (air temperature at 2 m (°C), soil temperature at 5 cm (°C), precipitation (mm), precipitation for 10 days (mm), air humidity (%) and Vapour pressure deficit (VPD)) at Aukstaitija monitoring station in 2016 April – October.

birch prefers similar sites as Scots pine and thus, a typical species composition is a Silver birch admixture in stands dominated by Scots pine or Norway spruce (Hynynen *et al.*, 2010).

In the hemi-boreal regions, mixtures of Scots pine, Norway spruce and Silver birch are common in poor or moderately fertile forest sites (Ge *et al.*, 2011). Predicted climate changes can impair these forests especially in soils of low water-holding capacity and susceptible to soil water deficit (Kellomäki *et al.*, 2008).

It is important to understand gas exchange response to environmental stress among tree species under changing climate conditions in hemi-boreal forests. Although we have basic information of growth characteristics of the 3 species native to Lithuania by observation, we should know their functional aspect of their growth traits under changing environment. This is necessary to make appropriate forest management decisions on tree species adaptation to changing climate.

The aim of this study was to compare gas exchange characteristics and chlorophyll fluorescence of the prevailing in Lithuania tree species: Scots pine, Norway spruce and Silver birch and to evaluate their physiological response to water stress.

We hypothesised that: (i) the gas exchange characteristics and chlorophyll fluorescence of the tree species differ under extreme environmental condition; (ii) Scots pine is more likely to be tolerant to different weather conditions, and (iii) Norway spruce is more likely to be sensitive to drought conditions.

2. Material and methods

2.1 Study site

The study was conducted in Aukstaitija National Park, located in the eastern part of Lithuania which belongs to hemi-boreal zone. Mixed conifer-deciduous forest dominated by Scots pine with admixture of Norway spruce and Silver birch prevail in the site. The mean age of pines trees was about 70 years, mean diameter (DBH) – 31.8 cm; mean height – 29.5 m. The mean age of spruce trees was about 50 years, mean diameter (DBH) – 26.8 cm; mean height – 25.6 m. The mean age of birch trees was about 70 years, mean diameter (DBH) – 33.4 cm; mean height – 30.5 m. The forests of *Pinetum vaccinio-myrtilosum* types prevail in the site. *Sorbus aucuparia*, *Frangula alnus* and *Juniperus communis*

were present in shrub layer. Evergreen dwarf shrubs *Vaccinium myrtillus*, *V. vitis-idaea* (coverage 15%) and herbs *Melampyrum pratense* (coverage 1%) dominated in the herb layer. *Pleurozium schreberi*, *Hylocomium splendens* and *Dicranum polysetum* (coverage 70%) dominated in the moss layer.

The average annual air temperature is $6.9 \pm 0.8^\circ\text{C}$, the average annual precipitation is 680 ± 100 mm. Growing season lasts 189 days on average (Augustaitis, 2011).

Soil type of site was *Haplic Arenosol*, water table was deeper than 2 m. Dry bulk density in upper soil layer (AB – 9–24 cm) was 1.43 g cm^{-3} , while in deeper soil layer (B1–25–80 cm) – 1.52 g cm^{-3} . Texture of the soil was characterized as sand (sand 89.8 – 92.1%, silt 4.6 – 6.2% and clay 3.3 – 4.0%). Base saturation ranged from 14.40 (upper soil layer) to 18.52 mekv kg^{-1} (deeper soil layer); pH_{KCl} – from 4.4 (at 15 cm soil depth) to 4.9 (at 50 cm soil depth); carbon content – from 1.11 to 0.56%; nitrogen content – from 0.036 to 0.030%; available potassium – from 98 to 79 mg kg^{-1} ; phosphorus – from 98 to 79 mg kg^{-1} ; Ca^{2+} – from 500 to 299 mg kg^{-1} , and Mg^{2+} – from 228 to 96 mg kg^{-1} .

2.2 Meteorological parameters, soil water potential, trees leaves/needle phenology and weather periods

The meteorological parameters were obtained from the Aukstaitija integrated European monitoring and Evaluation Programme (EMEP) monitoring station (Augustaitis, 2011; Augustaitis *et al.*, 2015). The following meteorological parameters were recorded: air temperature at 2 m ($^\circ\text{C}$), soil temperature at 5 cm ($^\circ\text{C}$), precipitation (mm), air humidity (%), Precipitation for 10 days before the measurements was calculated as an estimate of soil water content. Vapour pressure deficit (VPD) was calculated from temperature and vapour pressure of the air (Fig. 1).

Soil water potential was determined weekly at 20 cm and 40 cm soil depth using WATERMARK sensors (Fig. 2).

In 2016 deciduous early leaves of Silver birch started to unfold on 27 April and early leaves were fully developed on 8 of May. The leaves of Silver birch started to fall on 9 September and were fully fallen on 15 October. In 2016 needles of Scots pine started to unfold on 15 May and finished on 10 June.

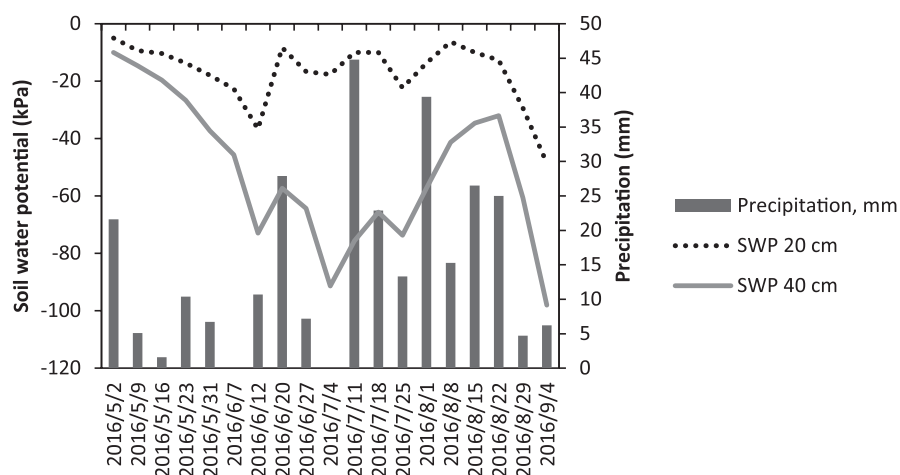


Fig. 2. Soil water potential (kPa) and weekly precipitation at Aukstaitija monitoring station in 2016 May – September. (SWP20 – soil water potential at 20 cm soil depth; SWP40 – soil water potential at 40 cm soil depth).

Table 1. Average diurnal meteorological parameters of weather period.

Date	Air temperature, 2 m (°C)	Soil temperature, 5 cm (°C)	PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Precipitation per 10 days before (mm)	Air humidity (%)	Vapor pressure deficit (VPD) (kPa)
20/05/2016	11.1	9.3	714	12.0	70.4	0.390
02/07/2016	22.8	16.6	908	0.0	41.4	1.624
27/07/2016	21.2	17.9	519	30.6	81.7	0.375
26/08/2016	18.2	15.8	790	14.7	77.8	0.464

Needles of Norway spruce started to unfold on 12 May and finished on 8 June.

The gas exchange and chlorophyll fluorescence measurements were performed in four weather periods with different meteorological conditions in the vegetation season of 2016: – the beginning of vegetation season (20/05/2016); – the middle of vegetation season (02/07/2016); – the middle of vegetation season (27/07/2016), and the end of vegetation season (26/08/2016) (Table 1).

2.3 Measurements of leaves/needles gas exchange

Gas exchange measurements were done using a LCPro+gas exchange system (ADC BioScientific, UK) with a standard 2.5×2.5 cm broadleaf chamber (area – 6.25 cm^2) for broadleaved trees and coniferous chamber (area – 100 cm^2) for coniferous tree species. We calculated mean projected area for pine and spruce needles in the coniferous chamber. The 5 twigs of the pine and spruce which were placed into the coniferous chamber were collected. We separated the needles from the twig and measured projection area for each twig separately using scanner and WinDias3 (Image analyses systems) software. We calculated the mean value for each species. We used conversion factor for projected area of pine and spruce needles considering needles shape (Cannel, 1982).

Gas exchange was recorded in 2016 in the end of May, in the beginning of July, in the end of July, and in the end of August (Table 1). Measurements were performed using current year intact leaves for Silver birch and one year old needles for coniferous species exposed to direct sunlight. During each measurement we used needles of the same age. We measured the three leaves/needles of the three individuals of the tree species on the same days between 12:00 and 14:00. The trees were selected at the canopy edge due to practical possibility to access leaves/needles exposed to direct sunlight. The crowns of the tree were at about 5-6 m from the ground and were reached using a ladder.

Measurements were made at saturating irradiance photosynthetic photon flux density PPFD ($1500 \mu\text{mol m}^{-2} \text{s}^{-1}$) and an ambient temperature, humidity and CO_2 concentration. Light-saturated photosynthetic rate (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$), transpiration rate (E , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), and intercellular CO_2 concentration (C_i , $\mu\text{mol mol}^{-1}$) were measured. Using the measured A , E , g_s and C_i values, the water use efficiency ($\text{WUE} = A/E$, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} / \text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), the intrinsic water use efficiency considering the stomatal mechanism ($i\text{WUE} = A/g_s$, $\mu\text{mol mol}^{-1}$), and the instantaneous carboxylation efficiency (A/C_i , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} / \mu\text{mol mol}^{-1}$) were calculated.

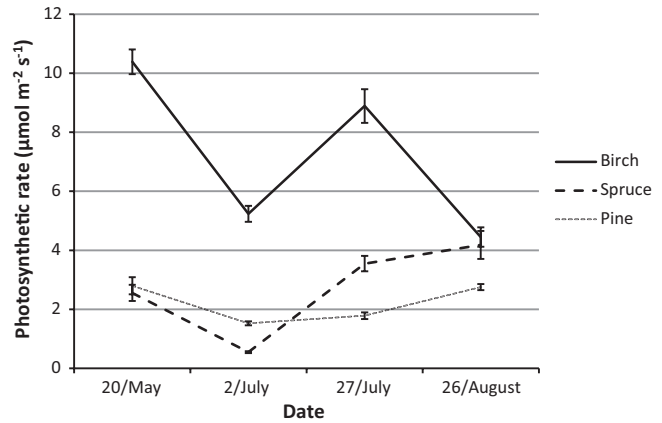


Fig. 3. Photosynthetic rates of the tree species in different weather periods (points are means and whiskers - standard errors).

During gas-exchange measurements the uniform leaf/needle photosynthesis and transpiration over the leaf/needle area was assumed (Terashima, 1992).

2.4 Measurements of chlorophyll fluorescence

The ratio of variable chlorophyll fluorescence to maximal chlorophyll fluorescence (F_v/F_m) of leaves/needles was measured with a portable fluorimeter (OS5p Modulated Chlorophyll Fluorimeter, Opti-Sciences, Inc. USA). Leaves/needles were acclimated to the dark for at least 20 min before the measurements using the leaf clip provided by the manufacturer. Chlorophyll fluorescence was measured on three leaves/needles of the three individuals of the tree species between 11:00 and 14:00 on the same days as gas exchange was measured. All measurements were performed on the same shoots of the trees.

2.5 Data analyses

ANOVA was used to compare parameters between different weather periods and tree species. The least significant difference method (LSD) was used to test the significant differences at level of $P < 0.05$. The Pearson correlation (r) was used to verify the relation between gas exchange and meteorological parameters. All statistical analyses were carried out using the STATISTICA 7.0 software package.

3. Results

The photosynthetic rate of Silver birch was higher (up to $10 \mu\text{mol m}^{-2} \text{s}^{-1}$) compared with coniferous species during

the whole vegetation period (Fig. 3). The photosynthetic rate of Silver birch was lowest during moderate drought weather conditions and in the end of vegetation season. All tree species showed lower photosynthetic rates during the moderate drought period. The largest reduction of photosynthetic rate during the moderate drought period was observed for Norway spruce. Norway spruce displayed higher photosynthetic rate compared with Scots pine during the warm humid weather period (Fig. 3). Photosynthetic rate of Silver birch and Scots pine but not that of Norway spruce correlated negatively with air and soil temperature (Table 2). Photosynthetic rate of Silver birch and Norway spruce correlated negatively, VPD and positively correlated with precipitation and air humidity. Photosynthetic

Table 2. Pearson correlation coefficients between gas exchange, chlorophyll fluorescence and meteorological parameters of the weather periods (Tm_2 m - air temperature during measurement, 2 m °C, S_tm_5 - soil temperature during measurement 5 cm °C, Hum - air humidity during measurement %, VPD - vapor pressure deficit during measurement kPa, Preci10d - precipitation per 10 days before measurement mm. *A* - photosynthetic rate, *E* - transpiration rate, *g_s* - stomatal conductance, *C_i* - intercellular CO₂ concentration, *A/C_i* - instantaneous carboxylation efficiency, WUE - water use efficiency, iWUE - intrinsic water-use efficiency, Fv/Fm - maximal chlorophyll fluorescence).

		Tm_2m	S_tm_5	Hum	VPD	Preci10d
<i>A</i>	Birch	-0.57*	-0.52*	0.57*	-0.59*	0.38*
	Pine	-0.58*	-0.43*	0.39*	-0.50*	0.03
	Spruce	-0.29	0.06	0.73*	-0.71*	0.69*
<i>E</i>	Birch	0.06	0.08	0.18	-0.18	0.47*
	Pine	0.34*	0.54*	0.42*	-0.25	0.68*
	Spruce	0.31*	0.56*	0.53*	-0.40*	0.73*
<i>g_s</i>	Birch	0.08	0.26	0.71*	-0.54*	0.88*
	Pine	-0.07	0.26	0.60*	-0.54*	0.68*
	Spruce	0.01	0.18	0.40*	-0.33*	0.48*
<i>C_i</i>	Birch	0.33*	0.67*	0.38*	-0.22	0.55*
	Pine	0.43*	0.63*	0.43*	-0.22	0.73*
	Spruce	0.71*	0.46*	-0.66*	0.75*	-0.42*
<i>A/C_i</i>	Birch	-0.65*	-0.69*	0.40*	-0.47*	0.14
	Pine	-0.62*	-0.59*	0.10	-0.28	-0.20
	Spruce	-0.42*	-0.09	0.65*	-0.67*	0.55*
WUE	Birch	-0.77*	-0.75*	0.50*	-0.60*	0.19
	Pine	-0.71*	-0.65*	0.12	-0.33*	-0.23
	Spruce	-0.86*	-0.69*	0.62*	-0.74*	0.28
iWUE	Birch	-0.48*	-0.80*	-0.36*	0.31*	-0.67*
	Pine	-0.41*	-0.69*	-0.45*	0.33*	-0.75*
	Spruce	-0.40*	-0.03	0.67*	-0.69*	0.58*
Fv/Fm	Birch	-0.21	-0.24	0.18	-0.18	0.10
	Pine	0.26	0.29	-0.06	0.14	0.15
	Spruce	-0.05	-0.07	-0.08	-0.07	0.08

* significance at *P*-0.05

rate of Scots pine did not correlate with precipitation, but it correlated negatively with VPD and correlated positively with humidity (Table 2).

Transpiration rate of Silver birch was higher (up to 2.6 mmol m⁻² s⁻¹) compared with coniferous species during the whole vegetation period (Fig. 4). The highest transpiration rate of Silver birch was observed during the humid weather period and it was 4–5 times higher than that of coniferous species. Transpiration rate of Norway spruce was significantly higher during the humid and warm weather periods. Transpiration rate of Scots pine remained similar during different weather periods (Fig. 4). Transpiration rate of Silver birch positively correlated only with precipitation. Transpiration rate of Scots pine and Norway spruce positively correlated with air and soil temperature, precipitation and humidity, transpiration rate of Norway spruce negatively correlated with VPD (Table 2).

Stomatal conductance of all tree species positively correlated with precipitation and humidity and negatively correlated with VPD (Table 2). Silver birch demonstrated higher stomatal conductance (*g_s*) compared with coniferous species. The highest stomatal conductance of Silver birch was during the humid

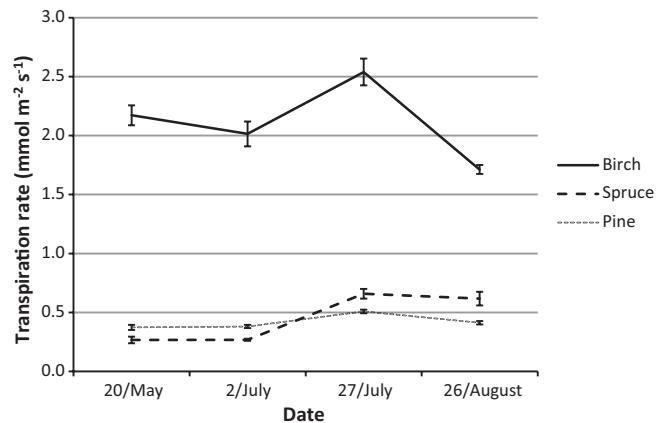


Fig. 4. Transpiration rates of the tree species in different weather periods (points are means and whiskers - standard errors).

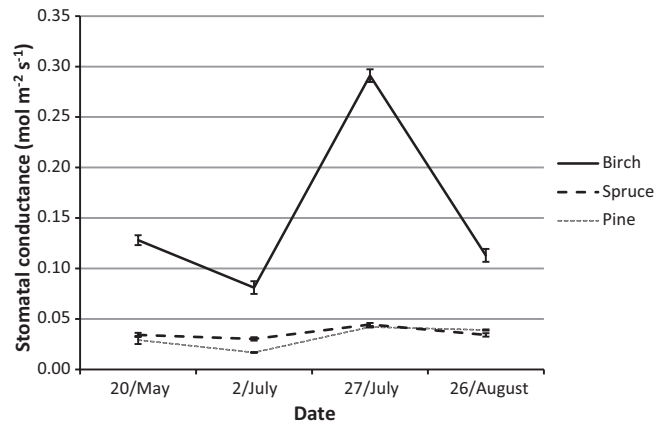


Fig. 5. Stomatal conductance of the tree species in different weather periods (points are means and whiskers - standard errors).

weather period. Both coniferous species displayed the lowest stomatal conductance during the drought weather period and the highest conductance during the humid weather period (Fig. 5). Norway spruce exhibited the lowest response of stomatal conductance to meteorological conditions.

Silver birch demonstrated the highest intercellular CO₂ concentration (C_i) during the whole vegetation season. Intercellular CO₂ concentration of Norway spruce was highest during the drought weather period. Scots pine demonstrated the highest intercellular CO₂ concentration during the humid weather period, probably because of increased stomatal conductance (Fig. 6). Intercellular CO₂ concentration of all species correlated positively with air and soil temperature (Table 2). Intercellular CO₂ concentration of Silver birch and Scots pine correlated positively with precipitation and humidity. Intercellular CO₂ concentration of Norway spruce correlated positively with VPD and correlated negatively with precipitation and humidity (Table 2). Such findings indicate a non-stomatal regulation of Norway spruce photosynthetic rate during moderate drought.

Silver birch demonstrated higher instantaneous carboxylation efficiency compared with coniferous species, except in the end of vegetation season (Fig. 7). Norway spruce displayed the

lowest instantaneous carboxylation efficiency during the drought weather period. Scots pine demonstrated the lowest instantaneous carboxylation efficiency during the humid weather period probably. Instantaneous carboxylation efficiency of Silver birch and Scots pine correlated negatively with air, soil temperature and VPD, but it did not correlate with precipitation. Instantaneous carboxylation efficiency of Scots pine correlated negatively with air and soil temperature, but it did not correlate with precipitation, humidity and VPD. Instantaneous carboxylation efficiency of Norway spruce correlated positively with precipitation and humidity, and correlated negatively with air temperature and VPD (Table 2). Instantaneous carboxylation efficiency of Norway spruce was sensitive to moisture conditions.

WUE of all tree species correlated negatively with air, soil temperature, and VPD. In general, coniferous species demonstrated higher WUE than Silver birch (Fig. 8). Silver birch demonstrated lower WUE during the moderate drought period due to reduced photosynthetic rate and during humid weather period due to increased transpiration rate. In the end of vegetation season Silver birch showed lower WUE efficiency because of degradation of the photosynthetic apparatus. Norway spruce displayed the lowest WUE efficiency during the drought weather period due to significantly reduced photosynthetic rate, while transpiration rate remained rather high. In the humid weather period and in the end of vegetation period WUE efficiency of Norway spruce was higher than in the drought period. Scot pine demonstrated higher WUE efficiency than Norway spruce during the drought weather period, but WUE efficiency of Scot pine was lower compared with Norway spruce in the humid weather period (Fig. 8). WUE of Norway spruce correlated positively with humidity (Table 2). Precipitation within 10 days before the measurements did not correlate with WUE of all tree species, suggesting that soil moisture had no effect on water use efficiency.

Intrinsic water use efficiency (iWUE), defined as the ratio between photosynthetic rate and stomatal conductance, demonstrated similar tendencies as described above for WUE (Fig. 9). IWUE of Silver birch and Scots pine correlated negatively with air, soil temperature, precipitation, and humidity

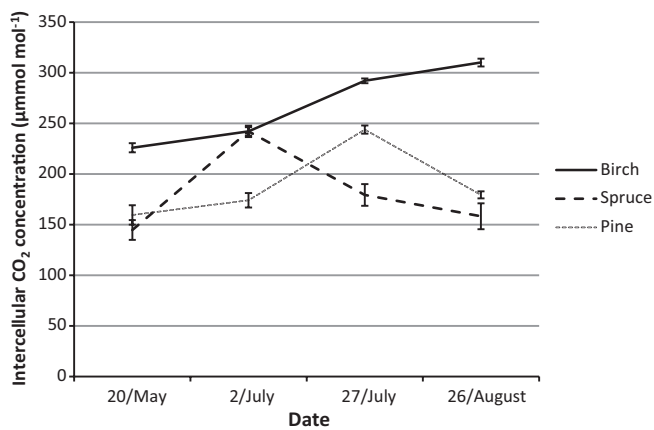


Fig. 6. Intercellular CO₂ concentration of the tree species in different weather periods (points are means and whiskers - standard errors).

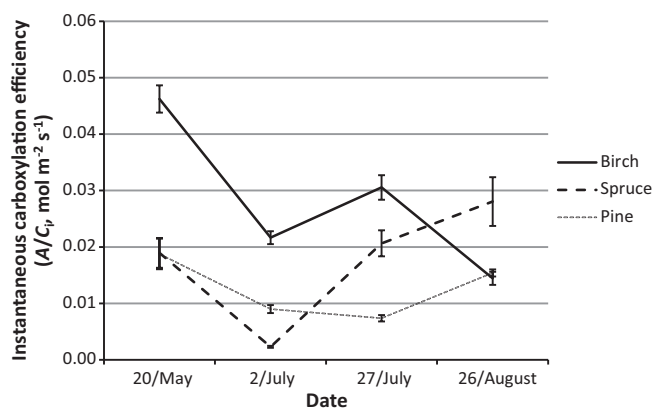


Fig. 7. Instantaneous carboxylation efficiency of the tree species in different weather periods (points are means and whiskers - standard errors).

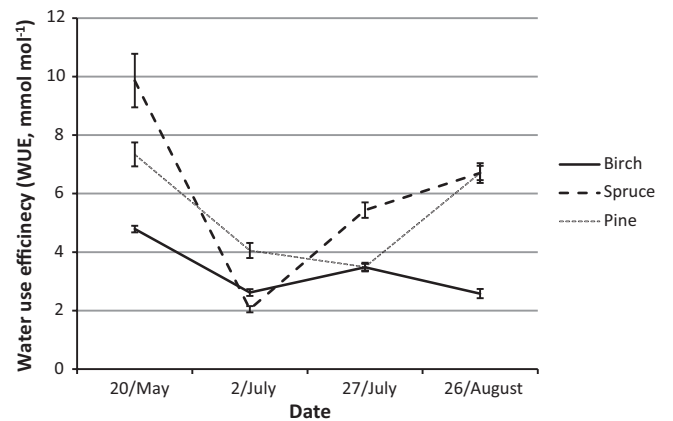


Fig. 8. Water use efficiency of the tree species in different weather periods (points are means and whiskers - standard errors).

and positively correlated with VPD (Table 2). Opposite correlations (except air temperature) were noted for iWUE of Norway spruce – it correlated negatively with VPD and correlated positively with precipitation and humidity (Table 2).

Maximal chlorophyll fluorescence (Fv/Fm) of all species was rather high and ranged from 0.764 to 0.834. There were no significant differences between species and weather periods, except for Silver birch which showed the lowest average value of Fv/Fm in the end of vegetation season (Fig. 10). Fv/Fm did not correlate with meteorological parameters (Table 2).

Intercorrelations among gas exchange and chlorophyll fluorescence parameters of tree species are shown in Table 3. Photosynthetic rate of all species correlated positively with stomatal conductance and correlated negatively with intercellular CO₂ concentration. Photosynthetic rate of Silver birch and Norway spruce but not of Scots pine correlated positively with transpiration rate. Photosynthetic rate of Silver birch correlated positively with Fv/Fm. All other gas exchange parameters did not correlate with Fv/Fm. Transpiration rate of all tree species correlated positively with stomatal conductance, but only transpiration rate of Scots pine correlated positively with intercellular CO₂ concentration.

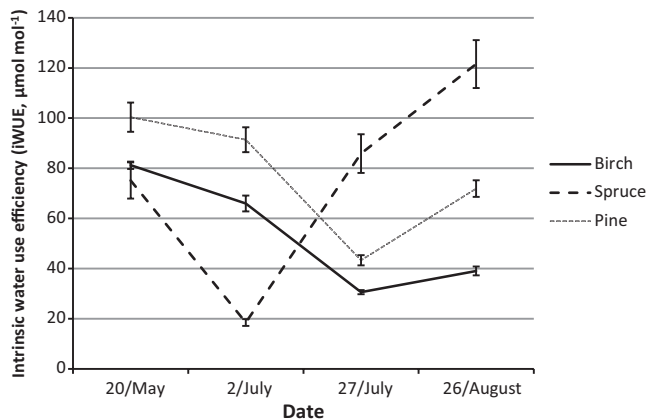


Fig. 9. Intrinsic water use efficiency of the tree species in different weather periods (points are means and whiskers - standard errors).

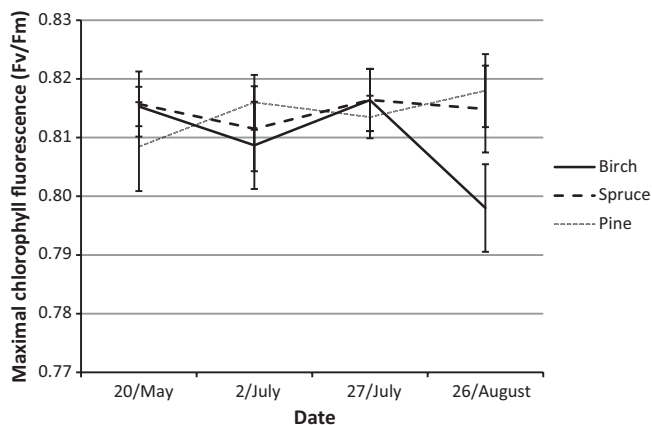


Fig. 10. Maximal chlorophyll fluorescence (Fv/Fm) of the tree species in different weather periods (points are means and whiskers - standard errors).

Table 3. Pearson correlation coefficients among gas exchange, and chlorophyll fluorescence parameters (*A* - photosynthetic rate, *E* - transpiration rate, *g_s* - stomatal conductance, *C_i* - intercellular CO₂ concentration, Fv/Fm - maximal chlorophyll fluorescence).

Parameters	<i>E</i>	<i>g_s</i>	<i>C_i</i>	Fv/Fm
Silver birch				
<i>A</i>	0.73*	0.54*	-0.47*	0.45*
<i>E</i>		0.78*	-0.10	0.31
<i>g_s</i>			0.31	0.25
<i>C_i</i>				-0.28
Scots pine				
<i>A</i>	0.09	0.54*	-0.54*	0.03
<i>E</i>		0.74*	0.59*	0.31
<i>g_s</i>			0.30	0.30
<i>C_i</i>				0.17
Norway spruce				
<i>A</i>	0.80*	0.47*	-0.67*	-0.04
<i>E</i>		0.54*	-0.18	-0.10
<i>g_s</i>			-0.09	-0.03
<i>C_i</i>				-0.10

* significance at < 0.05

4. Discussion

We found that all investigated tree species demonstrated reduced photosynthetic rates, lower stomatal conduction and transpiration rate under moderate drought conditions. Photosynthesis is one of the physiological processes most sensitive to drought stress. Plants can avoid drought stress by minimizing transpiration rate via stomatal closure. This, in turn, reduces internal CO₂ availability in the vicinity of the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), thus declining the plant photosynthetic rate. Reduced photosynthesis rate can be caused by stomatal regulation and other non-stomatal photosynthetic processes (Assmann, 1988; Lawlor, 2002; Lawlor and Tezara, 2009). Thus, trade-offs exist between water conservation and CO₂ assimilation rate for carbohydrate production. The decrease in photosynthetic rate during water stress has been shown to correlate with a reduction of Rubisco activity (Assmann, 1988; Anev *et al.*, 2016).

Stomatal closing is an early reaction of plants to water stress (Lawlor and Tezara, 2009). The positive correlation of photosynthesis with stomatal conductance and transpiration rate was considered to be an adaptive mechanism to water stress (Lawlor and Tezara, 2009). Besides stomatal closure, non-stomatal limitations on photosynthesis also occur during the periods of water stress. Stomatal conductance is a good indicator for determination of stomatal and non-stomatal limitations to photosynthesis (Xu and Zhou, 2008). As stomata close, the intercellular CO₂ concentration initially declines with increasing stress and then increases as drought becomes more severe.

We found that during the drought weather period intercellular CO₂ concentration of Norway spruce was higher compared to other weather periods. Such findings showed reduced

photosynthetic capacity due to non-stomatal regulation and proved sensitivity of Norway spruce to water stress. Lawlor (2002) reported reduced photosynthesis of Norway spruce under mild drought conditions, probably caused by a suppression of metabolism processes. The metabolic changes at drought conditions were responsible for low photosynthetic rate (Lawlor and Cornic, 2002). Under these conditions drought can impair ATP synthesis and thus ATP limited regeneration of RuBP. More severe drought leads to a reduced photochemical activity (Lawlor and Cornic, 2002).

The instantaneous carboxylation efficiency can be considered as the estimate of Rubisco activity under stress conditions (Niinemets *et al.*, 2009). High intercellular CO₂ concentration associated with a low stomatal conductance indicate a decrease in the instantaneous carboxylation efficiency. In our study, instantaneous carboxylation efficiency of tree species was lower during the drought weather period. The highest decrease in instantaneous carboxylation efficiency was demonstrated by Norway spruce due to significantly reduced photosynthetic rate, while instantaneous carboxylation efficiency of Scots pine reacted less to different weather conditions.

Stomata partial closing and consequently diminished transpiration rate ensures some carbon fixation and increased efficiency of water use (Yordanov *et al.*, 2000; Lawlor and Tezara, 2009).

WUE can serve as a water stress indicator for mature trees. WUE of trees is increasing with the extent of water stress (Anev *et al.*, 2016). Our study demonstrated lower water use efficiency of investigated tree species during the moderate drought period mainly due to the reduced photosynthetic rate. Other studies showed that in the early stage of water stress, the stomatal conductance decreases faster than the photosynthetic rate, resulting in increased WUE (Gilbert *et al.*, 2011; Edwards *et al.*, 2012; Anev *et al.*, 2016).

Severe drought can cause photosynthesis photoinhibition which occurs under conditions of almost complete stomata closure (Flexas and Medrano, 2002). Plant photosynthetic photoinhibition can be assessed by chlorophyll fluorescence. Chlorophyll fluorescence is a non-destructive efficient method to assess activity of plant photosystem II (PSII), which drives the light-phase of photosynthesis (Baker and Rosenqvist, 2004). One of the most widely used parameters is a maximal chlorophyll fluorescence (Fv/Fm), an indicator of photoinhibition (Ogaya and Peñuelas, 2003) and its value reaches 0.84. For healthy plants of various genera (Fv/Fm) value is 0.80–0.84. According to Maxwell and Johnson (2000), values of less than 0.75 indicate a state of plant stress.

We found no significant changes in Fv/Fm during different weather periods. During the study period, tree species did not experience severe drought stress and photoinhibition probably did not occur. Only Silver birch showed the lowest mean value of Fv/Fm in the end of vegetation season probably due to the photosynthetic apparatus started to degrade (Flexas and Medrano, 2002; Lawlor and Tezara, 2009).

Other studies have also shown Fv/Fm to be rather insensitive to changes occurring under conditions of low or moderate stress (Sofa *et al.*, 2008). In the experiment of four-year-old seedlings

of Norway spruce grown under semi-controlled conditions with three watering regimes, Fv/Fm was not sensitive to low or moderate drought (Ditmarova *et al.*, 2009).

We found that tree species reacted differently to weather conditions. Differences in stomatal response to drought may occur among species with different ecological strategies for controlling water relations under drought conditions. The isohydric behaviour was reported for Norway spruce, Scots pine and Silver birch (Tardieu and Simonneau, 1998; Uddling *et al.*, 2004; McDowell *et al.*, 2008; Hartmann, 2011). Plants that possess better stomatal regulation are more tolerant to drought. Our findings showed the lowest reaction of stomatal conductance to meteorological conditions for Norway spruce compared with other tree species. Photosynthetic rate of Norway spruce reacted more sensitively to precipitation and humidity compared with Scots pine and Silver birch. With regard to transpiration rate, Silver birch was the least sensitive to temperature and moisture, while Norway spruce was the most sensitive to moisture conditions. Norway spruce displayed lowest instantaneous carboxylation efficiency during the drought weather period due to significantly reduced photosynthetic rate. Instantaneous carboxylation efficiency of Norway spruce was sensitive to soil moisture conditions.

Ge *et al.* (2011) found different species responses to water deficit in mixed spruce-pine-birch and monoculture forests. Reduced precipitation and elevated temperatures had only small effects on Scots pine and birch but large impact on Norway spruce in boreal forest in Finland (Briceño-Elizondo *et al.*, 2006). Their findings supported our results on Norway spruce sensitivity to drought conditions.

In conclusion, our study showed that the investigated tree species reacted differently to weather conditions. Under moderate drought conditions all tree species demonstrated reduced photosynthetic rates, lower stomatal conduction transpiration rates, WUE and instantaneous carboxylation efficiency. No significant changes of Fv/Fm among species were detected during different weather conditions. The Scots pine demonstrated the highest tolerance whereas Norway spruce highest sensitivity to drought conditions. The Silver birch was the least sensitive to temperature and humidity conditions. If drought condition would continue, vegetation will be modified from spruce dominate to birch or pine.

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