

Research Article

SPECIAL ISSUE: Scaling Effects Regulating Plant Response to Global Change

Responses of sap flow, leaf gas exchange and growth of hybrid aspen to elevated atmospheric humidity under field conditions

Aigar Niglas¹, Priit Kupper¹, Arvo Tullus^{1,2} and Arne Sellin^{1*}¹ Institute of Ecology and Earth Sciences, University of Tartu, Lai 40, 51005 Tartu, Estonia² Institute of Forestry and Rural Engineering, Estonian University of Life Sciences, Kreutzwaldi 5, 51014 Tartu, Estonia

Received: 13 January 2014; Accepted: 29 April 2014; Published: 15 May 2014

Associate Editor: Tim J. Brodribb

Citation: Niglas A, Kupper P, Tullus A, Sellin A. 2014. Responses of sap flow, leaf gas exchange and growth of hybrid aspen to elevated atmospheric humidity under field conditions. *AoB PLANTS* 6: plu021; doi:10.1093/aobpla/plu021

Abstract. An increase in average air temperature and frequency of rain events is predicted for higher latitudes by the end of the 21st century, accompanied by a probable rise in air humidity. We currently lack knowledge on how forest trees acclimate to rising air humidity in temperate climates. We analysed the leaf gas exchange, sap flow and growth characteristics of hybrid aspen (*Populus tremula* × *P. tremuloides*) trees growing at ambient and artificially elevated air humidity in an experimental forest plantation situated in the hemiboreal vegetation zone. Humidification manipulation did not affect the photosynthetic capacity of plants, but did affect stomatal responses: trees growing at elevated air humidity had higher stomatal conductance at saturating photosynthetically active radiation ($g_{s\ sat}$) and lower intrinsic water-use efficiency (IWUE). Reduced stomatal limitation of photosynthesis in trees grown at elevated air humidity allowed slightly higher net photosynthesis and relative current-year height increments than in trees at ambient air humidity. Tree responses suggest a mitigating effect of higher air humidity on trees under mild water stress. At the same time, trees at higher air humidity demonstrated a reduced sensitivity of IWUE to factors inducing stomatal closure and a steeper decline in canopy conductance in response to water deficit, implying higher dehydration risk. Despite the mitigating impact of increased air humidity under moderate drought, a future rise in atmospheric humidity at high latitudes may be disadvantageous for trees during weather extremes and represents a potential threat in hemiboreal forest ecosystems.

Keywords: Atmospheric humidity; canopy conductance; climate change; net photosynthesis; photosynthetic capacity; relative stomatal limitation; stomatal conductance; water-use efficiency.

* Corresponding author's e-mail address: arne.sellin@ut.ee

Published by Oxford University Press on behalf of the Annals of Botany Company.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

With rapid increases in global industrial development, fossil fuel use and changing land-use practices, atmospheric CO₂ concentration ([CO₂]) is expected to double within the 21st century. This increase will result in global climate changes: global mean water vapour concentration, evaporation and precipitation rates, as well as global mean surface temperature are projected to increase during the 21st century (IPCC 2007). These changing climate factors along with rising [CO₂] affect the physiological performance of plants: CO₂ assimilation, transpiration, stomatal conductance (g_s) and ultimately plant growth and productivity.

The impact of the most common consequences of climate change—drought, high temperature and high atmospheric vapour pressure deficit (VPD)—on photosynthesis and water use in C₃ plants has been quite well studied, because the occurrence of extreme temperatures, soil water deficit and high VPD, as well as their interactions, alters the physical properties and yield of plants, which are important to agriculture and forestry (Fletcher et al. 2007; Guha et al. 2010; Estrada-Campuzano et al. 2012; Kuster et al. 2013; Li et al. 2013; Sapeta et al. 2013). Considerably less is known of the effect of increasing atmospheric humidity on plants. Increases in precipitation are considered very likely at high latitudes in the long-term perspective (IPCC 2007). Precipitation is predicted to increase in northern Europe, especially in winter, and to decrease in southern and central Europe in summer (Räisänen et al. 2004). There might also be fewer dry days at higher latitudes by the end of the 21st century (IPCC 2007). Increasing rainfall frequency results in higher relative air humidity at local or regional scales.

The leaves of plants grown at high relative humidity (RH) have larger stomata, larger stomatal pore aperture and length, and significantly lower stomatal density due to larger epidermal cells than in plants grown at moderate RH (Torre et al. 2003; Nejad and Van Meeteren 2005; Arve et al. 2013). Therefore, decreasing VPD may lead to increased stomatal conductance and to a consequent increase in transpiration in some plant species grown at high RH (Pospíšilová 1996; Fordham et al. 2001; Nejad and Van Meeteren 2005). Nevertheless, most findings suggest that a decrease in VPD generally leads to decreased steady-state leaf transpiration or sap flux density in a wide range of tree species from different habitats (Pataki et al. 1998; Meinzer 2003; Bovard et al. 2005; Hölscher et al. 2005). Our previous studies have demonstrated decreased sap flux density in response to increased air humidity in silver birch (*Betula pendula*) and hybrid aspen (*Populus tremula* × *P. tremuloides*) trees in moist summers (Kupper et al. 2011).

Growing at high RH not only alters stomatal morphology, but stomatal functioning as well (Fanourakis et al. 2010, 2011). It is known that RH is a key environmental factor mediating changes in stomatal sensitivity to CO₂ (Talbot et al. 2003). Moreover, RH affects stomatal response to water availability and drought. Plants grown at high RH are less hydrosensitive than plants grown at moderate RH: stomata of high-RH-grown leaves are less sensitive to decreases in leaf water potential than moderate-RH-grown leaves, and the homogeneity, speed and degree of stomatal closure are less in high-RH-grown plants (Nejad and Van Meeteren 2005; Rezaei Nejad et al. 2006; Rezaei Nejad and Van Meeteren 2008). Therefore, plants developed under moderate RH are able to retain higher water status due to more efficient stomatal control. Arve et al. (2013) revealed that stomata developed under high RH respond to neither darkness nor drought, but remain open. Thus, high RH may even override the signals given by darkness. The stomata of plants growing in naturally waterlogged soil are also less sensitive to decreasing VPD than those of plants growing in well-drained soil (Sellin 2001).

High RH does not change only the stomatal characteristics of plants. Our previous experiments with silver birch and hybrid aspen have shown that elevated atmospheric RH lowers leaf nutritional status by altering nutrient movement via mass flow in soil and lowering nutrient transfer through xylem flow into leaves (Tullus et al. 2012a; Sellin et al. 2013). The changes in leaf nutrient content and P:N ratio in turn cause a decline in photosynthetic capacity and ultimately changes in tree growth rate.

Experiments on stomatal responses to air humidity and plant stress resistance are typically carried out in greenhouses or growth chambers with seedlings or saplings growing in pots. The objective of the present study was to investigate how artificially increased RH during leaf development affects the sap flow, stomatal responses and photosynthetic parameters of hybrid aspen (*P. tremula* × *P. tremuloides*) under free-air conditions. Hybrid aspen is a fast-growing deciduous tree species suitable for short-rotation forestry in the relatively cold climate of northern Europe (Tullus et al. 2012b). Our aim was to test the following hypotheses. (i) Trees grown at higher atmospheric humidity have higher stomatal conductance and lower water-use efficiency (WUE) than control trees. (ii) The photosynthetic capacity of leaves developed in humid air is lower because of reduced nitrogen uptake due to lower transpirational flux density. (iii) Plants grown in a more humid atmosphere are unable to adjust their WUE quickly because of acclimation to lower VPD or possible stomatal malfunction.

Methods

Study area and sample trees

Studies were performed on hybrid aspen (*P. tremula* × *P. tremuloides*) saplings growing in an experimental forest plantation at the free-air humidity manipulation (FAHM) site, situated at Rõka village (58°24'N, 27°29'E, 40–48 m ASL) in eastern Estonia, representing a hemiboreal vegetation zone. The long-term average annual precipitation in the region is 650 mm and the average temperature is 17.0 °C in July and –6.7 °C in January. In the study year (2011) drought conditions prevailed in June and July (Fig. 1). The growing season lasts 175–180 days from mid-April to October. The soil is a fertile endogenic mollic planosol (WRB) with an A-horizon thickness of 27 cm. Total nitrogen content is 0.11–0.14 %, C/N ratio is 11.4 and pH is 5.7–6.3.

The study site, established on an abandoned agricultural field in 2006–07, is a fenced area of 2.7 ha containing nine circular experimental plots (diameter 14 m) planted with hybrid aspen and silver birch (*B. pendula*) and surrounded by a buffer zone. One-year-old micropropagated hybrid aspen plants were planted in the experimental area in the autumn of 2006. The stand density in the buffer zone is 2500 trees ha⁻¹, and in the experimental plots, 10 000 trees ha⁻¹. The computer-operated FAHM system, based on an integrated approach of two different technologies—a misting technique to atomize/vaporize water and FACE-like technology to mix humidified air inside the plots—enables RH of the air to increase by up to 18 % over the ambient level during the humidification treatment, depending on the wind speed inside the experimental stand. The humidification is applied during daytime 6 days a week throughout the growing period if ambient RH is <75 % and mean wind speed is <4 m s⁻¹. As a long-term average, RH is 7–8 % greater in humidified plots (H treatment) than in control plots (C treatment). A detailed description of the FAHM site

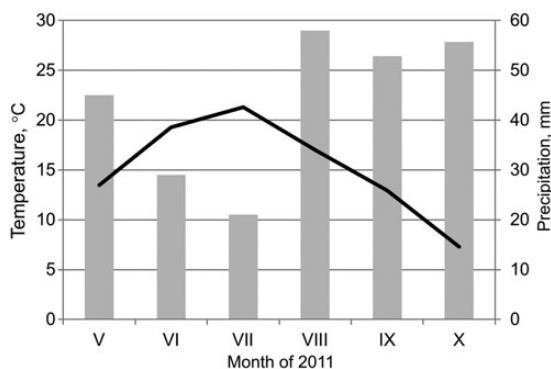


Figure 1. Weather data in the growing period of 2011: the dark line indicates monthly average air temperature, and the grey bars indicate monthly precipitation.

and technical setup is presented in Kupper et al. (2011). The treatment began in June 2008; sap flow and gas exchange were measured in the summer months of 2011. Soil water potential (Ψ_s) was recorded at depths of 15 and 30 cm with EQ2 equitensiometers (Delta-T Devices, Burwell, UK) in eight replications per plot. The daily average Ψ_s varied from June to August and was ~25 % higher in the humidification treatment (Fig. 2). The air temperature (T_a) and RH were measured 1.5–3.5 m above the ground with 2–4 HMP45A sensors (Vaisala, Helsinki, Finland) per plot. Sensor readings were collected every 1 min and stored as 10-min average values with a data logger (DL2e; Delta-T Devices). Air VPD was calculated from T_a , saturated vapour pressure and RH. The daily average VPD in the humidification treatment was 15 % lower than the control in the summer of 2011 (Fig. 3).

Sap flow measurements

Xylem sap flow in the stems of sample trees was measured with FLOW4 sap flow systems (Dynamax Inc.,

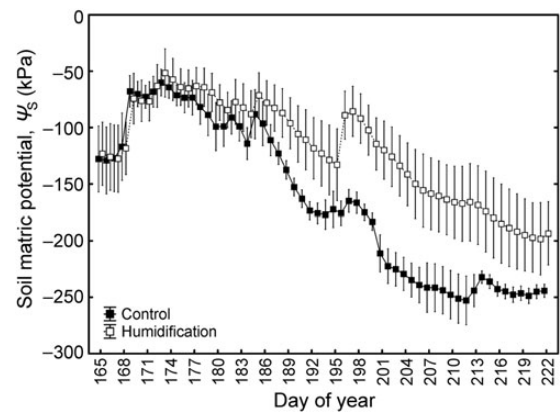


Figure 2. Daily average values of soil water potential at a depth of 15–30 cm in control and humidification plots from June to August in 2011. Scale bars denote SEM.

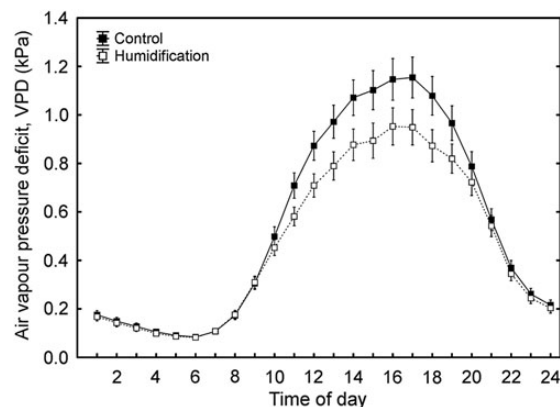


Figure 3. Hourly average values of air VPD in control and humidification plots from June to August in 2011. Scale bars denote SEM.

Houston, TX, USA). Six trees from control plots (C1, C2, C4) and four trees from humidification plots (H1, H2) were fitted with sap flow gauges (SGB35-WS) and sampled episodically from June to August 2011. Sap flow data were recorded every 1 min and stored as 30-min averages. Sap flux density (F ; $\text{g m}^{-2} \text{h}^{-1}$) was calculated as sap flow divided by whole-tree foliage area estimated by mean sapwood-to-leaf-area ratios (Huber value, HV) measured in nine **C** ($3.08 \times 10^{-4} \text{ m}^2 \text{ m}^{-2}$) and nine **H** ($3.09 \times 10^{-4} \text{ m}^2 \text{ m}^{-2}$) trees using destructive sampling. Foliage area, measured with a LI 3100C optical area meter (LI-COR Biosciences, Lincoln, NE, USA), was on average 31 % greater in **C** trees compared with **H** trees. Whole-tree canopy conductance to water vapour (g_c ; $\text{mmol m}^{-2} \text{ s}^{-1}$) was computed from the data of sap flux density ($\text{mmol m}^{-2} \text{ s}^{-1}$) using a simplified Penman–Monteith equation (Komatsu et al. 2006; Sellin and Lubenets 2010):

$$g_c = \frac{F \times P}{\text{VPD}}, \quad (1)$$

where P is atmospheric pressure (kPa) and VPD is air vapour pressure deficit (kPa).

Gas exchange measurements

We sampled gasometrically nine trees (mean height 3.8 m) from **C** plots and nine trees (mean height 3.3 m) from **H** plots (i.e. three trees per sample plot) for 1 month, from mid-July to mid-August. Measurements were performed on rainless misting-free days on intact fully expanded leaves *in situ* with a portable photosynthesis system LCpro+ (ADC BioScientific, Great Amwell, UK) at constant air humidity (13 mbar), CO_2 concentration ($C_a = 360 \mu\text{mol mol}^{-1}$) and temperature of the leaf chamber (25°C). Leaf-to-air vapour pressure difference was relatively similar in the two treatments: on average 2.12 kPa for **C** plants and 1.99 kPa for **H** plants. To generate photosynthetic light response curves (A/Q curves), four leaves per tree were sampled from the middle part of the crown with an instrument equipped with an LED light source. The measurements started with photosynthetically active radiation (PAR) at $1196 \mu\text{mol m}^{-2} \text{ s}^{-1}$, then decreased stepwise to $9 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and increased stepwise from 1196 to $1803 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Intrinsic water-use efficiency, expressed as the ratio of net photosynthesis (A_n) to stomatal conductance to water vapour (g_s), was determined at two levels of irradiance: at 400–600 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ when IWUE was usually at a maximum (IWUE_{max}) and at light intensities corresponding to full sunlight (IWUE_{sat}; $Q \geq 1400 \mu\text{mol m}^{-2} \text{ s}^{-1}$).

The response of net photosynthesis to varying intercellular CO_2 concentration (C_i)— A/C_i curves—was also

determined on intact leaves (four leaves per tree) *in situ* at constant air humidity (13 mbar), temperature of the leaf chamber (25°C) and at saturating irradiance ($1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$). External CO_2 concentration (C_a) was supplied in 11 steps, decreasing from 360 to $60 \mu\text{mol mol}^{-1}$ and then increasing from 450 to $1600 \mu\text{mol mol}^{-1}$. In addition to IWUE_{max} and IWUE_{sat} calculated from the data of A/Q curves, IWUE_{in} (initial IWUE) was determined using initial values of the A/Q and A/C_i sequences when external $[\text{CO}_2]$ was $360 \mu\text{mol mol}^{-1}$.

Tree growth assessment

Tree height (H , cm) and stem diameter at 30-cm height (D , mm) of all aspen trees growing at three **C** and three **H** plots were measured before and after the 2011 growing season. H was measured with a telescopic Nedo mEssfix-S measuring rod (Nedo GmbH & Co.KG, Dornstetten, Germany) and stem diameter with a LIMIT digital caliper (Luna AB, Alingsås, Sweden). Current annual increment of the trees (ΔH , ΔD) was estimated as the difference between the two measurements. Relative increment (ΔH_{rel} , ΔD_{rel}) was expressed as the ratio of ΔH and ΔD to their respective characteristics at the beginning of the growing season. The ratio of $H : D$ was defined as tree slenderness (S).

Data analysis

Statistical data analysis was carried out using Statistica, Ver. 7.1 (StatSoft Inc., Tulsa, OK, USA). Repeated-measures analysis of variance (ANOVA) was used to compare the sap flux density (F) and canopy conductance to water vapour (g_c) between trees from the control and the misting treatment. The daily averages of F and g_c were analysed altogether on 31 days from 1000 to 1700 h from 14 June to 7 August 2011 (DOY: 165–176, 197–201, 206–219). Linear regression analysis was carried out to estimate relationships between F , g_c , VPD and Ψ_s . The normality of the regression residuals was checked using the Shapiro–Wilk test.

The gasometric data were analysed with Photosyn Assistant, Ver. 1.2 software (Dundee Scientific, Dundee, UK). The A/Q curves were fitted as a non-rectangular hyperbola expressed as a quadratic equation by Prioul and Chartier (1977). The initial slope of the curve expresses the apparent quantum efficiency (ϕ), whereas the X and Y axes intercepts, respectively, correspond to the light compensation point (Q_{comp}) and apparent dark respiration (R_d), and the upper asymptote approximates the light-saturated rate of photosynthesis (A_{max}). An additional parameter—convexity (θ)—is required to describe the rate of bending between the linear increase and the maximum value. Sub-stomatal cavity CO_2 concentration

(C_i) was calculated using the model of von Caemmerer and Farquhar (1981).

The A/C_i curves were analysed according to the biochemical model proposed by Farquhar et al. (1980), and subsequently modified by Harley and Sharkey (1991) and Harley et al. (1992). This model enables estimation of the CO_2 compensation point (Γ), the maximum rate of carboxylation by Rubisco ($V_{C\max}$), the PAR-saturated rate of electron transport (J_{\max}) and the rate of triose phosphate utilization (V_{TPU}), which indicates the availability of inorganic phosphorus for the Calvin cycle (Sharkey 1985). The relative stomatal limitation on photosynthesis

Table 1. Soil water potential (kPa) estimates of the experimental plots: Ψ_{S_mean} , mean across the growing season; Ψ_{S_Q25} , lower quartile; Ψ_{S_Q75} , upper quartile.

Plot	Ψ_{S_mean}	Ψ_{S_Q25}	Ψ_{S_Q75}
C1	-197	-240	-152
C2	-191	-217	-177
C4	-196	-217	-185
H1	-56	-76	-33
H2	-194	-221	-175
H4	-124	-151	-97

(L_s), an estimate of the proportion of the reduction in photosynthesis attributable to CO_2 diffusion between the atmosphere and intercellular space, was calculated from the A/C_i curves as follows (Farquhar and Sharkey 1982; Tissue et al. 2005; Huang et al. 2008):

$$L_s = \left(1 - \frac{A_n}{A_0}\right) 100, \quad (2)$$

where A_n is the net photosynthetic rate at normal C_a ($360 \mu\text{mol mol}^{-1}$) and A_0 is the photosynthetic rate when C_i ($= 360 \mu\text{mol mol}^{-1}$) equals C_a . Under these conditions, A_0 is the rate of photosynthesis that would occur if there were no diffusive limitation to CO_2 transfer through stomatal pores. The effect of humidification on gas exchange parameters was analysed by applying a nested analysis of variance with fixed factors of 'Treatment', 'Experimental plot' and 'Soil water potential' (a continuous variable), the second nested in the first. As plant physiological traits were more strongly related to the soil water potential measured at 30-cm depth (Ψ_{30}), we used this parameter as an index of soil water status. Because of drought development during the measurement period, we divided the datasets of both treatments into two groups according to Ψ_{30} (< -204 kPa for drier

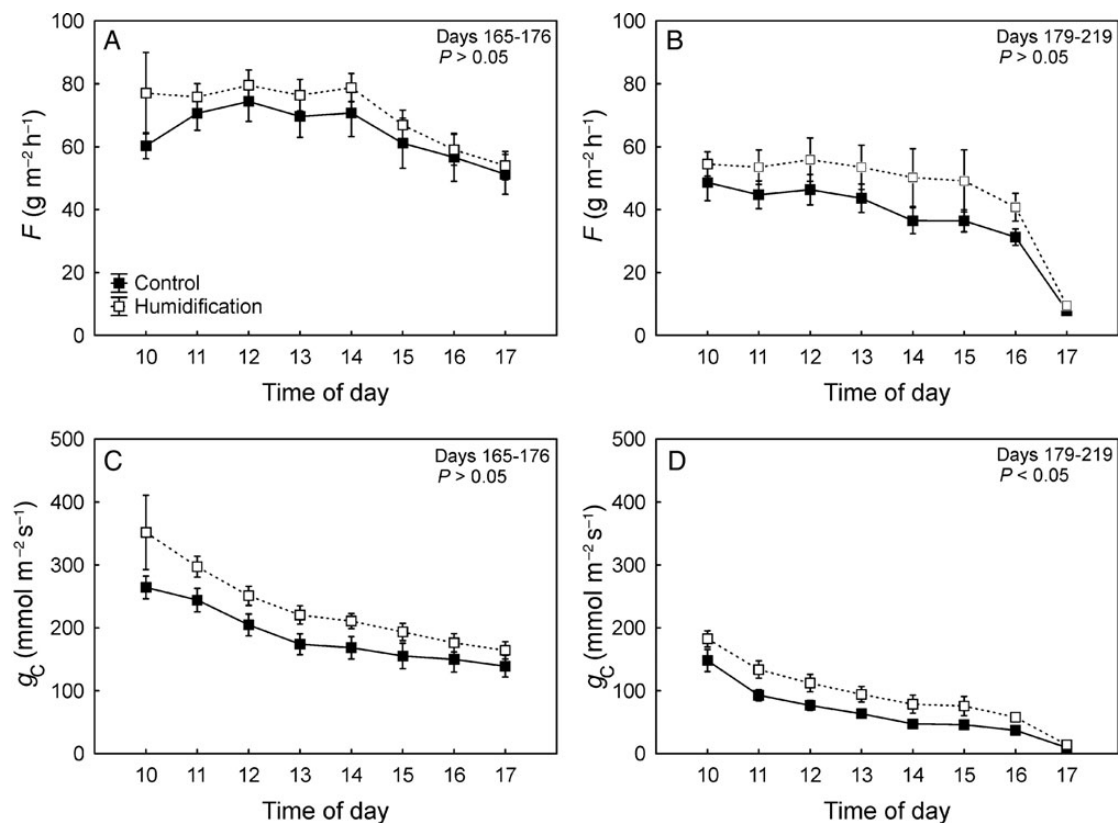


Figure 4. Daily average values of sap flux density (F) and canopy conductance to water vapour (g_c) in control and humidification plots during mist fumigation from June 14 to June 25 (DOY: 165–176; A and C) and July 15 to August 7 (DOY: 179–219; B and D), 2011. Scale bars denote SEM.

soil and ≥ -204 kPa for moister soil in **C** plots; < -163 and ≥ 163 kPa in **H** plots, respectively) and analysed gas exchange data also separately for these conditions.

Student's *t*-test was applied to estimate the treatment effect on the growth characteristics of individual trees across all experimental plots. Analysis of variance models

were used to study the effects of 'Treatment' and 'Experimental plot' (nested in treatment) or 'Treatment' and 'Soil water potential' as a continuous covariate on the growth characteristics. Means and upper and lower quartiles of daily average soil water potentials (Ψ_{S_mean} , Ψ_{S_Q25} , Ψ_{S_Q75}) across the growing season were used as covariates in separate models (Table 1). When exploring the variance of total and relative growth increment in 2011, tree size (*H* or *D* at the end of the previous growing season) was included as a covariate. Type IV sums of squares were used in the calculations; post hoc mean comparisons were conducted using Tukey's HSD test.

Results

Sap flux density and canopy conductance

Although canopy conductance (g_c) was significantly higher (22 %; $P < 0.05$) under humidification across the whole study period, the difference between the treatments was statistically insignificant for days 165–176 (Fig. 4C) when the soil water potential did not differ between the **C** and **H** plots (Fig. 2). Also the sap flux density in the **H** treatment was on average 13 % higher than in the **C** treatment, although the difference was not significant (Fig. 4A and B). g_c decreased with increasing VPD ($P < 0.001$) in both the **C** and **H** plots; the response patterns were completely coincident and the slopes of the respective regression lines did not differ between the treatments (Fig. 5A). g_c also decreased with decreasing Ψ_S ($P < 0.001$), while the treatments demonstrated contrasting sensitivities ($dg_c/d\Psi_S$) to developing soil water deficit—the corresponding slopes were 0.94 and 3.01 for control and humidified trees, respectively (Fig. 5B).

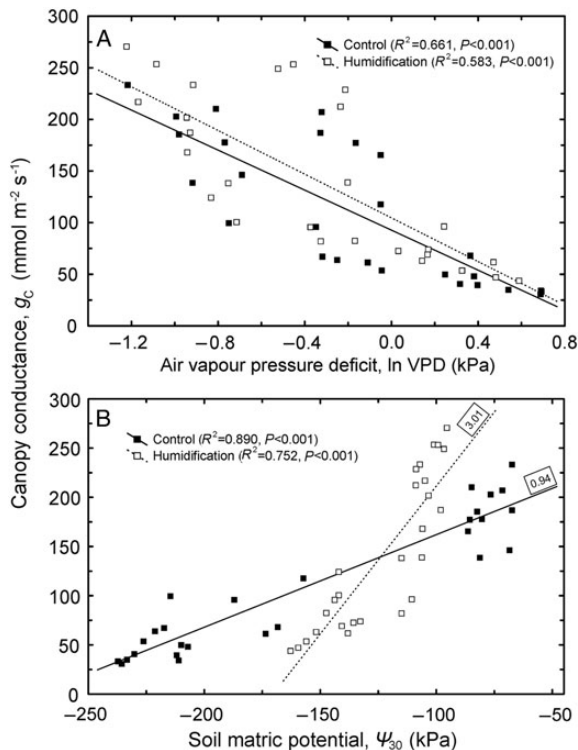


Figure 5. Variation in daily average canopy conductance to water vapour (g_c) depending on atmospheric VPD (A) and bulk soil water potential at a depth of 30 cm (Ψ_{30} ; B). The numbers by the regression lines indicate the respective slopes.

Table 2. Leaf gas exchange characteristics of hybrid aspen growing under control and humidification treatment. Each value is the mean \pm SE; the means are compared with Tukey's test. NS, not statistically significant.

Parameter	Treatment		Significance level (<i>P</i>)
	Control \pm SE	Humidification \pm SE	
A_n ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	10.5 \pm 0.4	11.3 \pm 0.4	NS
g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	0.22 \pm 0.01	0.28 \pm 0.01	0.002
$g_{s \text{ sat}}$ ($\text{mol m}^{-2} \text{s}^{-1}$)	0.19 \pm 0.02	0.25 \pm 0.02	0.040
IWUE_{in} ($\mu\text{mol mol}^{-1}$)	53.4 \pm 1.5	45.1 \pm 1.5	<0.001
IWUE_{max} ($\mu\text{mol mol}^{-1}$)	62.3 \pm 2.29	54.4 \pm 2.19	0.015
IWUE_{sat} ($\mu\text{mol mol}^{-1}$)	56.8 \pm 1.88	48.81 \pm 2.19	0.008
L_s (%)	41.3 \pm 1.01	37.7 \pm 1.01	0.016
A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	12.9 \pm 0.6	12.9 \pm 0.6	NS
$V_{C \text{ max}}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	56.5 \pm 2.9	59.9 \pm 2.7	NS
J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	173 \pm 10	196 \pm 11	NS

Leaf gas exchange

Average net photosynthesis (A_n) tended to be slightly greater in trees growing at elevated atmospheric humidity than those grown at ambient RH, although the treatment means did not differ statistically throughout the experiment (Table 2). Analysis of variance revealed that the humidity treatment affected stomatal response, but not leaf photosynthetic traits (Tables 2 and 3). Specifically, there were significant differences in means of stomatal conductance to water vapour measured at saturating

PAR ($g_{s \text{ sat}}$) and IWUE between the treatments: $g_{s \text{ sat}}$ was 32 % higher and $IWUE_{in}$ 16 % lower in the H treatment than in C trees (Table 2).

The data analysis revealed that soil water availability affected the gas exchange parameters differently within the treatments. A_n and $g_{s \text{ sat}}$ in the H treatment were significantly greater under moist soil conditions ($12.45 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $0.300 \text{ mol m}^{-2} \text{s}^{-1}$, respectively) than under drier conditions ($9.78 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $0.192 \text{ mol m}^{-2} \text{s}^{-1}$, respectively; Fig. 6A and B). Initial

Table 3. Effects of treatment, plot and soil water potential at a depth of 30 cm (Ψ_{30}) on gas exchange characteristics. NS, not statistically significant.

Characteristic	Factor	Significance level (P)
A_n ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Treatment	NS
	Plot (nested in treatment)	<0.001
	Ψ_{30}	0.013
g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	Treatment	NS
	Plot (nested in treatment)	0.002
	Ψ_{30}	NS
$g_{s \text{ sat}}$ ($\text{mol m}^{-2} \text{s}^{-1}$)	Treatment	0.027
	Plot (nested in treatment)	0.022
	Ψ_{30}	NS
$IWUE_{in}$ ($\mu\text{mol mol}^{-1}$)	Treatment	0.013
	Plot (nested in treatment)	0.002
	Ψ_{30}	NS
$IWUE_{max}$ ($\mu\text{mol mol}^{-1}$)	Treatment	<0.001
	Plot (nested in treatment)	0.005
	Ψ_{30}	0.003
$IWUE_{sat}$ ($\mu\text{mol mol}^{-1}$)	Treatment	<0.001
	Plot (nested in treatment)	0.012
	Ψ_{30}	0.007
A_{max}	Treatment	NS
	Plot (nested in treatment)	NS
	Ψ_{30}	NS
$V_{C \text{ max}}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Treatment	NS
	Plot (nested in treatment)	NS
	Ψ_{30}	0.045
J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Treatment	NS
	Plot (nested in treatment)	NS
	Ψ_{30}	0.007
L_s (%)	Treatment	NS
	Plot (nested in treatment)	<0.001
	Ψ_{30}	0.015

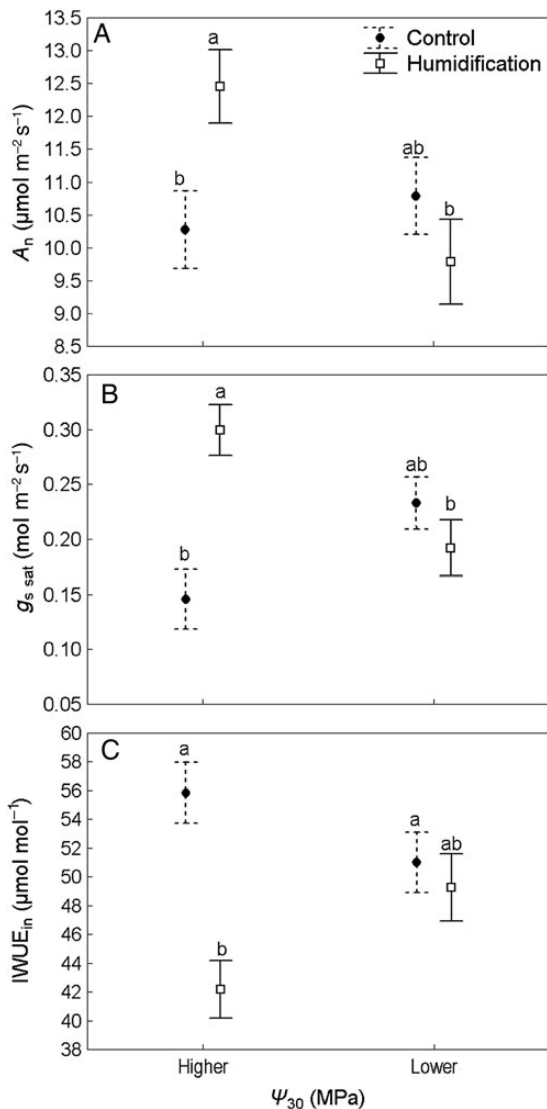


Figure 6. Means of net photosynthesis (A_n ; A), stomatal conductance to water vapour at saturating PAR ($g_{s \text{ sat}}$; B) and intrinsic water-use efficiency (IWUE_{in}; C) of control (closed circles) and humidified trees (open squares) depending on soil water status. Values are means \pm SE; different letters denote statistically significant ($P < 0.05$) differences.

intrinsic water-use efficiency also differed with respect to soil conditions: it was lower under moist soil conditions than under drier conditions (44.2 and 49.3 $\mu\text{mol mol}^{-1}$, respectively). There were no differences in A_n , $g_{s \text{ sat}}$ and IWUE_{in} with respect to soil moisture in C plots (Fig. 6). It is important to notice that g_s , $g_{s \text{ sat}}$, IWUE_{in} and A_{max} did not depend on Ψ_{30} (as a continuous variable) across the whole dataset (Table 3).

Photosynthesis was strongly associated with g_s in both treatments: $R^2 = 0.84$, $P < 0.001$ in C plots and $R^2 = 0.79$, $P < 0.001$ in H plots. There was an inverse linear relationship between IWUE_{in} and C_i/C_a ($R^2 = 0.73$, $P < 0.001$),

while the slopes of the corresponding regressions did not differ between the treatments ($P > 0.05$). A_n increased with rising CO_2 concentration (C_a), with significantly ($P < 0.001$) steeper response in the H treatment ($\beta = 53.5$; $R^2 = 0.82$, $P < 0.001$) than in the control ($\beta = 45.5$; $R^2 = 0.74$, $P < 0.001$). There were no differences in the $A_n = f(C_i)$ slopes between the treatments. g_s decreased with increasing C_a , but the responses did not differ between the treatments. As a consequence, IWUE_{in} rose with C_a ; the slope for control trees was greater than that for humidified trees ($P < 0.001$; Fig. 7).

The maximum rate of carboxylation by Rubisco ($V_{C \text{ max}}$) and the maximum rate of electron transport (J_{max}) did not differ between the treatments across the whole dataset; ANOVA revealed only an effect of soil water status on these parameters (Table 3). When the data were analysed separately in two groups (moist versus dry soil conditions), significant differences between the means of $V_{C \text{ max}}$ and J_{max} became evident only for the humidification treatment—both parameters were higher ($P < 0.001$ for both parameters) in moist soil. No variation with the soil conditions was detected in $V_{C \text{ max}}$ and J_{max} in the control trees (Fig. 8A and B). Regression analysis revealed a positive relationship between $V_{C \text{ max}}$ ($R^2 = 0.168$, $P < 0.001$) and J_{max} ($R^2 = 0.151$, $P < 0.01$) and Ψ_{30} , as well as between $V_{C \text{ max}}$ and J_{max} ($R^2 = 0.85$, $P < 0.001$) across both treatments.

The mean values of relative stomatal limitation of photosynthesis (L_s) were lower in trees grown at elevated RH than in C trees—37.9 and 41.3 %, respectively ($P < 0.05$; Table 2), although ANOVA did not establish any significant effect of the treatment (Table 3). Net photosynthesis was negatively correlated with L_s in control trees ($R^2 = 0.15$, $P = 0.03$), but the relationship lacked in the humidification treatment ($P = 0.23$). We found no differences in L_s with respect to soil water status in any treatment separately (Fig. 8C).

Impact on growth rate

Saplings of hybrid aspen growing in H plots were significantly shorter and had narrower stems (Table 4), regardless of whether sample plot or soil water potential was included as confounding factors in the models (Table 5). The absolute and relative growth increments in 2011 were either unaffected by treatment or significantly greater in H plots (Tables 4 and 5). This was more pronounced when $\Psi_{S \text{ mean}}$ or $\Psi_{S \text{ Q25}}$ was used as a covariate in ANOVA models, although using $\Psi_{S \text{ Q25}}$ yielded slightly better approximations than the two other soil water potential estimates (Table 5). Slenderness (S) was unaffected by treatment, but varied significantly among the experimental plots.

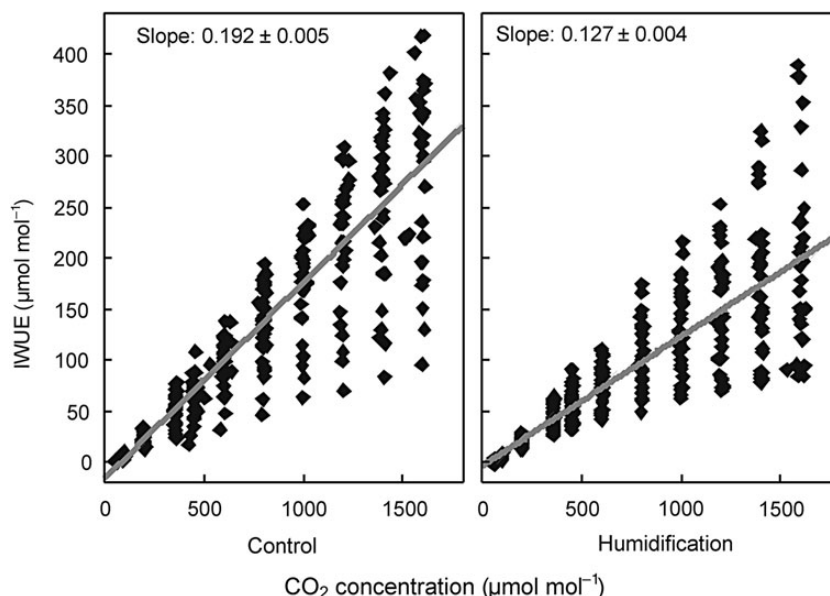


Figure 7. Photosynthetic WUE versus external CO_2 concentration in control and humidification treatment.

Discussion

Effects on sap flow and gas exchange

The sap flux density in hybrid aspen trees changed considerably compared with that at the same experimental site in previous summers (Kupper et al. 2011; Tullus et al. 2012a). F in control plots was significantly greater than that in the humidification treatment during the rainy summer of 2009 (Kupper et al. 2011). The same tendency (not significant) was observed in the drier summer of 2010 (Tullus et al. 2012a). However, our current results demonstrate higher (although not statistically significant) sap flux densities in trees growing in the humidification plots (Fig. 4). The canopy conductance to water vapour in the **H** treatment was greater ($P < 0.05$) across the whole study period. This discrepancy is attributable to relatively low soil water potential in the control treatment owing to the very dry summer: Ψ_s did not rise over -80 kPa in July. The total precipitation during the 2011 growing season (May–October) was 261 mm (Fig. 1), 42 % less than the average of the three previous years (452 mm). As the air humidity manipulation did not affect sapwood-to-leaf-area ratio (HV), the ~ 30 % greater leaf area of control trees was responsible for greater transpirational water losses causing faster depletion of soil water reserves and a greater decline in Ψ_s despite lower overall sap flux densities in **C** plots (Fig. 4A and B).

The response of canopy conductance to changes in VPD did not vary between the treatments, suggesting that stomatal sensitivity to atmospheric evaporative demand was not affected by the experimental manipulation. In contrast to that, g_c decreased much faster in response

to falling Ψ_s in the **H** treatment than in the control (Fig. 5). The differential response of g_c to decreasing soil water availability is probably mediated by plant hydraulic conductance (Cohen and Naor 2002; Domec et al. 2009). Hydraulic measurements performed on aspen trees in 2010 revealed that both soil-to-branch and leaf hydraulic conductances expressed per unit leaf area were smaller in humidified trees, although growing in moister soil (A. Sellin, unpubl. res.). Under conditions of soil water deficit the lower hydraulic capacity probably becomes a crucial factor for **H** trees, limiting leaf water supply and inducing a steep decline in canopy conductance. Furthermore, an experiment with silver birch revealed that a rapid water deficit in **H** plants led to a faster decrease in hydraulic conductance—responsible for liquid water supply—compared with the decrease in g_s , which limits water losses, and exposed plants to a greater risk of dehydration (Sellin et al. 2014).

Lowering A_n and g_s and increasing IWUE are typical responses to water stress in plant species with a drought avoidance strategy. When plants encounter a soil water deficit, abscisic acid (ABA) is synthesized in the roots and translocated to the leaf through the transpiration stream (Assmann and Shimazaki 1999); higher concentrations of ABA in leaves drive mechanisms leading to a decrease in g_s and an increase in WUE (Liu et al. 2005). Pantin et al. (2013) propose that ABA promotes stomatal closure in two ways—via its widely known biochemical effect on guard cells and via an indirect hydraulic effect through a decrease in leaf hydraulic conductance. Maintaining stable gas exchange attributes during drought development means that a plant either possesses a drought

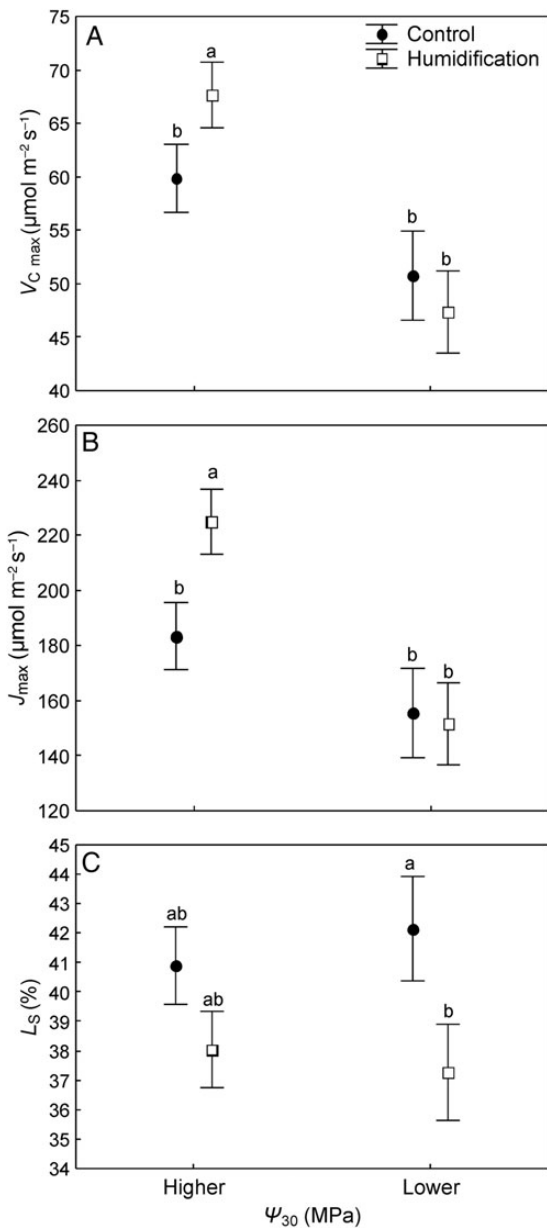


Figure 8. Means of maximum rate of carboxylation by Rubisco ($V_{C \max}$; A), PAR-saturated rate of electron transport (J_{\max} ; B) and relative stomatal limitation to photosynthesis (L_s ; C) of control (closed circles) and humidified trees (open squares) depending on soil water status. Values are means \pm SE; different letters denote statistically significant ($P < 0.05$) differences.

tolerance strategy or lacks adaptations with respect to drought. *Populus tremula*, one of the parent species, is known to act as a drought avoider (Possen et al. 2011). In our case, the lack of variation in gas exchange characteristics in C plots with respect to soil water status (Fig. 6) and modification of gas exchange in H plots by altering water availability can be explained by the lower Ψ_s values in the C treatment (i.e. long-term effects mediated by ABA).

Regardless of the difference in Ψ_s between the treatments, there was still an effect of the humidity manipulation on $g_{s \text{ sat}}$ and IWUE (Table 3). In fact, the differences in leaf gas exchange between the C and H plots are attributable to the combined effects of soil water availability and increased atmospheric humidity. As such, our first hypothesis is supported by the experiment: growing at higher RH increases stomatal conductance in trees while lowering photosynthetic WUE (Table 2), while the effect is largely mediated by changes in soil water status.

Soil water potential influenced both $V_{C \max}$ and J_{\max} in hybrid aspen (Table 3). Grassi et al. (2005) found a positive relationship between $V_{C \max}$ and Ψ_s in oak and ash trees during summer, as in hybrid aspen in this study. A simulation by Grassi and Magnani (2005) indicated that 30–40 % of the biochemical limitation could be attributed to a reduction in leaf nitrogen content during droughty summers. Plant photosynthetic capacity and leaf N content expressed per leaf area are positively correlated (Grassi et al. 2005; van de Weg et al. 2012). Previous work at the FAHM experimental site has shown that rising RH reduces the water flux in silver birch (Kupper et al. 2011) and alters the nutritional status of leaves, leading to a decline in photosynthetic capacity (Sellin et al. 2013). In this experiment, humidification increased rather than decreased water flux through the trees (Fig. 4), which explains why the biochemical capacity of photosynthesis was unaffected by the manipulation, but by changes in Ψ_s (Table 3). The second hypothesis concerning reduced photosynthetic capacity of leaves due to the expectedly smaller N uptake in humidified trees remained unconfirmed. The absence of an impact of air humidity manipulation on photosynthetic machinery of hybrid aspen was also supported by chlorophyll fluorescence measurements performed in the droughty summer of 2012 (A. Niglas, unpubl. res.).

High RH can affect stomatal sensitivity by changing stomatal morphology: plants grown at higher RH have larger stomata that close to a lesser extent when leaves dry (Giday et al. 2013). In addition, long-term acclimation to high RH during growth increases heterogeneity in stomatal response characteristics to short-term exposure to stomatal closure-inducing factors (Nejad and Van Meeteren 2005). Experiments showing differences in stomatal sensitivity and morphology between plants grown at high and low RH have been carried out under stable/controlled environmental conditions. The conditions before a leaf is fully expanded are important determinants on whether stomatal closure capacity is affected by leaf dehydration and RH. Moreover, the degree of stomatal adaptation in expanding leaves depends on the duration and timing of the exposure to high RH (Fanourakis et al. 2011). The present study was performed under field

Table 4. Comparison (*t*-test) of mean (\pm SE) growth parameters of individual trees growing in humidified (H) and control (C) plots. NS, not statistically significant.

Growth characteristic	C	H	t-stat	P
Height (cm)	454 \pm 5.8	428 \pm 5.8	3.06	0.002
Diameter of stem at 30-cm height (mm)	32.6 \pm 0.7	29.5 \pm 0.6	3.39	<0.001
Height increment of the current year (cm year ⁻¹)	111 \pm 2.3	117 \pm 2.7	-1.70	NS
Relative height increment of the current year	0.34 \pm 0.01	0.39 \pm 0.01	-3.46	<0.001
Diameter increment of the current year (mm year ⁻¹)	8.9 \pm 0.3	8.5 \pm 0.2	1.14	NS
Relative diameter increment of the current year	0.38 \pm 0.01	0.41 \pm 0.01	-1.65	NS
Slenderness (height-to-diameter ratio)	14.4 \pm 0.2	14.8 \pm 0.2	-1.97	0.051

Table 5. Results from ANOVA models describing the effect of tree size (i.e. the value of the respective parameter before the start of the growing season, C_{t-1}), treatment (*T*), plot and soil water potential (Ψ_{S_mean} , Ψ_{S_Q25} , Ψ_{S_Q75}) on the growth parameters.

Factors		Response variables						
		H	D	ΔH	ΔH_{rel}	ΔD	ΔD_{rel}	S
Model 1								
C_{t-1}	P	-	-	0.824	<0.001	<0.001	0.766	-
<i>T</i>	P	<0.001	<0.001	0.074	0.247	0.069	0.086	0.051
Plot (<i>T</i>)	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.015
	Adj. R^2	0.29	0.13	0.09	0.37	0.37	0.16	0.03
Model 2								
C_{t-1}	P	-	-	0.802	<0.001	<0.001	0.006	-
<i>T</i>	P	<0.001	<0.001	0.019	0.013	0.041	0.017	0.668
Ψ_{S_mean}	P	<0.001	0.157	<0.001	<0.001	<0.001	<0.001	0.005
	Adj. R^2	0.08	0.03	0.09	0.36	0.32	0.09	0.03
Model 3								
C_{t-1}	P	-	-	0.880	<0.001	<0.001	0.004	-
<i>T</i>	P	<0.001	<0.001	0.013	<0.001	0.049	0.018	0.800
Ψ_{S_Q25}	P	<0.001	0.081	<0.001	<0.001	<0.001	<0.001	0.010
	Adj. R^2	0.09	0.04	0.10	0.37	0.32	0.09	0.03
Model 4								
C_{t-1}	P	-	-	0.684	<0.001	<0.001	0.008	-
<i>T</i>	P	<0.001	0.002	0.083	0.077	0.075	0.052	0.622
Ψ_{S_Q75}	P	<0.001	0.351	<0.001	<0.001	<0.001	<0.001	0.002
	Adj. R^2	0.07	0.03	0.07	0.35	0.32	0.08	0.04

conditions with natural diurnal fluctuations of RH; misting was applied when ambient RH was <75 % and could be increased to as much as 18 % (versus 60 and 95 % of RH in Fanourakis et al. 2011). Our data suggest that stomatal sensitivity to atmospheric VPD remained unaffected in saplings of hybrid aspen. Although we did not explore

stomatal dimensions, we presume that differences in morphology and the putative morphological effect on stomatal sensitivity were rather minor as our trees grew *in natura*, in both diurnally and seasonally variable environments, under conditions requiring flexible stomatal adjustment.

The findings that stomatal conductance decreases and photosynthesis increases with rising external CO₂ level are well-known phenomena (reviewed by Araujo et al. 2011). A steeper A_n response to C_a in H plots is attributable to higher stomatal conductance (evidenced by g_{s,max}) due to leaf development under lower VPD (Table 2). This is indirectly confirmed also by the negative correlation between A_n and L_S in the C treatment.

Initial intrinsic water-use efficiency responded more sensitively to C_a in C plots than in H plots (Fig. 7), testifying once more to the effect of elevated atmospheric humidity on leaf gas exchange. Because high IWUE is advantageous to plants under drought conditions, a slower response in high-RH-grown trees to changing ambient conditions may be disadvantageous in the case of abrupt climatic fluctuations becoming more frequent in the future (Easterling et al. 2000); these plants are not able to adjust their water use as quickly as plants grown in drier air and experience greater water loss. Thus, our results support the third hypothesis on the capacity of plants to modify WUE under changing environmental conditions, albeit not directly tested with respect to air humidity.

Consequences on tree growth

The above-ground growth response of aspen trees to humidification in 2011 demonstrated some trends in contrast to those observed in previous years (Tullus et al. 2012a). However, the positive effect of humidification was detectable only in current-year height increments, while overall dimensions remained smaller in H plots, where hybrid aspen trees had grown slower than in C plots in the two previous experimental years (Tullus et al. 2012a). The inverse growth response is also attributable to dry weather conditions prevailing in summer 2011. Generally Ψ_5 or experimental plot was the more significant factor influencing tree growth response than humidity manipulation. One must take into account that the two factors— Ψ_5 and treatment—are partly interrelated, as transpirational flux through trees was lower in H plots (Fig. 4A; see also Kupper et al. 2011 and Tullus et al. 2012a) and more water was retained in the soil (Fig. 2). However, the humidity manipulation also had an impact on growth when considering the effect of Ψ_5 ; thus, the humidification effect on tree growth was clearly not due solely to altered soil water availability. In average or rainy years, when soil water does not limit growth, lowered transpiration hinders nutrient uptake by trees in H plots (Tullus et al. 2012a), especially for nutrients migrating to the roots with mass flow in soil. Under these conditions increased atmospheric humidity does not improve the growth rate of hybrid aspen. Sellin et al. (2013) also showed that humidification treatment lowers

the photosynthetic capacity and growth rate of silver birch in moist summers. In dry years, when soil water availability limits growth, the impact of this mechanism is obviously irrelevant.

Conclusions

The current study demonstrates that higher air humidity mitigates the effect of low soil water availability on broadleaved trees during dry years by reducing stomatal limitation to photosynthesis, allowing higher net photosynthetic rates and supporting higher growth rates (relative height growth). At the same time, higher RH increases the sensitivity of canopy conductance to water deficit and reduces the responsiveness of IWUE to factors inducing stomatal closure. The present and our earlier results (Tullus et al. 2012a; Sellin et al. 2013, 2014) imply that a future rise in atmospheric humidity at high latitudes may be disadvantageous in evenly rainy/humid years and expose trees to a higher dehydration risk during weather extremes, although mitigating the impact of soil water deficit under moderate drought.

Sources of Funding

This study was supported by the Estonian Science Foundation (Grant no. 8333), by the Estonian Ministry of Education and Research (target financing project SF0180025s12), and by the EU through the European Social Fund (Mobilitas postdoctoral grant MJD 257) and the European Regional Development Fund (Project No. 3.2.0802.11-0043 'BioAtmos' and Centre of Excellence in Environmental Adaptation).

Contributions by the Authors

A.N. and A.S. designed and performed the experiment, and wrote the manuscript. P.K. and A.T. performed the experiment, analysed the data and revised the paper. All authors read and approved the final manuscript.

Conflicts of Interest Statement

None declared.

Acknowledgements

We are grateful to Kristina Lubenets for installing sap flow gauges, Jaak Sõber for operating the FAHM humidification system and providing weather data, and Robert Szavakovats for language revision.

Literature Cited

- Araújo WL, Fernie AR, Nunes-Nesi A. 2011. Control of stomatal aperture: a renaissance of the old guard. *Plant Signaling & Behavior* **6**: 1305–1311.
- Arve LE, Terfa MT, Gislørød HR, Olsen JE, Torre S. 2013. High relative air humidity and continuous light reduce stomata functionality by affecting the ABA regulation in rose leaves. *Plant, Cell and Environment* **36**:382–392.
- Assmann SM, Shimazaki KI. 1999. The multisensory guard cell. Stomatal responses to blue light and abscisic acid. *Plant Physiology* **119**:809–815.
- Bovard BD, Curtis PS, Vogel CS, Su HB, Schmid HP. 2005. Environmental controls on sap flow in a northern hardwood forest. *Tree Physiology* **25**:31–38.
- Cohen S, Naor A. 2002. The effect of three rootstocks on water use, canopy conductance and hydraulic parameters of apple trees and predicting canopy from hydraulic conductance. *Plant, Cell and Environment* **25**:17–28.
- Domec J-C, Noormets A, King JS, Sun G, McNulty SG, Gavazzi MJ, Boggs JL, Treasure EA. 2009. Decoupling the influence of leaf and root hydraulic conductances on stomatal conductance and its sensitivity to vapour pressure deficit as soil dries in a drained loblolly pine plantation. *Plant, Cell and Environment* **32**: 980–991.
- Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO. 2000. Climate extremes: observations, modeling, and impacts. *Science* **289**:2068–2074.
- Estrada-Campuzano G, Slafer GA, Miralles DJ. 2012. Differences in yield, biomass and their components between triticale and wheat grown under contrasting water and nitrogen environments. *Field Crops Research* **128**:167–179.
- Fanourakis D, Matkaris N, Heuvelink E, Carvalho SMP. 2010. Effect of relative air humidity on the stomatal functionality in fully developed leaves. *Acta Horticulturae* **870**:83–88.
- Fanourakis D, Carvalho SMP, Almeida DPF, Heuvelink E. 2011. Avoiding high relative air humidity during critical stages of leaf ontogeny is decisive for stomatal functioning. *Physiologia Plantarum* **142**:274–286.
- Farquhar GD, Sharkey TD. 1982. Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology* **33**:317–345.
- Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* **149**:78–90.
- Fletcher AL, Sinclair TR, Allen LH Jr. 2007. Transpiration responses to vapor pressure deficit in well watered 'slow-wilting' and commercial soybean. *Environmental and Experimental Botany* **61**: 145–151.
- Fordham MC, Harrison-Murray RS, Knight L, Evered CE. 2001. Effects of leaf wetting and high humidity on stomatal function in leafy cuttings and intact plants of *Corylus maxima*. *Physiologia Plantarum* **113**:233–240.
- Giday H, Kjaer KH, Fanourakis D, Ottosen CO. 2013. Smaller stomata require less severe leaf drying to close: a case study in *Rosa hybrida*. *Journal of Plant Physiology* **170**:1309–1316.
- Grassi G, Magnani F. 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant, Cell and Environment* **28**:834–849.
- Grassi G, Vicinelli E, Ponti F, Cantoni L, Magnani F. 2005. Seasonal and interannual variability of photosynthetic capacity in relation to leaf nitrogen in a deciduous forest plantation in northern Italy. *Tree Physiology* **25**:349–360.
- Guha A, Rasineni GK, Reddy AR. 2010. Drought tolerance in mulberry (*Morus* spp.): a physiological approach with insights into growth dynamics and leaf yield production. *Experimental Agriculture* **46**: 471–488.
- Harley PC, Sharkey TD. 1991. An improved model of C₃ photosynthesis at high CO₂: reversed O₂ sensitivity explained by lack of glycerate reentry into the chloroplast. *Photosynthesis Research* **27**:169–178.
- Harley PC, Loreto F, Marco GD, Sharkey TD. 1992. Theoretical considerations when estimating the mesophyll conductance to CO₂ flux by analysis of the response of photosynthesis to CO₂. *Plant Physiology* **98**:1429–1436.
- Hölscher D, Koch O, Korn S, Leuschner C. 2005. Sap flux of five co-occurring tree species in a temperate broad-leaved forest during seasonal soil drought. *Trees* **19**:628–637.
- Huang Z, Xu Z, Blumfield TJ, Bubba K. 2008. Variations in relative stomatal and biochemical limitations to photosynthesis in a young blackbutt (*Eucalyptus pilularis*) plantation subjected to different weed control regimes. *Tree Physiology* **28**:997–1005.
- IPCC. 2007. *Climate change 2007: the physical science basis*. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, eds. Cambridge: Cambridge University Press.
- Komatsu H, Kang Y, Kume T, Yoshifuji N, Hotta N. 2006. Transpiration from a *Cryptomeria japonica* plantation, Part 2: Responses of canopy conductance to meteorological factors. *Hydrological Processes* **20**:1321–1334.
- Kopper P, Söber J, Sellin A, Löhmus K, Tullus A, Räm O, Lubenets K, Tulva I, Uri V, Zobel M, Kull O, Söber A. 2011. An experimental facility for free air humidity manipulation (FAHM) can alter water flux through deciduous tree canopy. *Environmental and Experimental Botany* **72**:432–438.
- Kuster TM, Schleppei P, Hu B, Schulin R, Günthardt-Goerg MS. 2013. Nitrogen dynamics in oak model ecosystems subjected to air warming and drought on two different soils. *Plant Biology* **15**(Suppl. 1):220–229.
- Li D, Liu H, Qiao Y, Wang Y, Cai Z, Dong B, Shi C, Liu Y, Li X, Liu M. 2013. Effects of elevated CO₂ on the growth, seed yield, and water use efficiency of soybean (*Glycine max* (L.) Merr.) under drought stress. *Agricultural Water Management* **129**:105–112.
- Liu F, Jensen CR, Shahanzari A, Andersen MN, Jacobsen SE. 2005. ABA regulated stomatal control and photosynthetic water use efficiency of potato (*Solanum tuberosum* L.) during progressive soil drying. *Plant Science* **168**:831–836.
- Meinzer FC. 2003. Functional convergence in plant responses to the environment. *Oecologia* **134**:1–11.
- Nejad AR, Van Meeteren U. 2005. Stomatal response characteristics of *Tradescantia virginiana* grown at high relative air humidity. *Physiologia Plantarum* **125**:324–332.
- Pantin F, Monnet F, Jannaud D, Costa JM, Renaud J, Muller B, Simonneau T, Genty B. 2013. The dual effect of abscisic acid on stomata. *New Phytologist* **197**:65–72.
- Pataki DE, Oren R, Katul G, Sigmon J. 1998. Canopy conductance of *Pinus taeda*, *Liquidambar styraciflua* and *Quercus phellos* under

- varying atmospheric and soil water conditions. *Tree Physiology* **18**:307–315.
- Pospišilová J. 1996. Effect of air humidity on the development of functional stomatal apparatus. *Biologia Plantarum* **38**:197–204.
- Possen BJHM, Oksanen E, Rousi M, Ruhanen H, Ahonen V, Tervahauta A, Heinonen J, Heiskanen J, Kärenlampi S, Vapaavuori E. 2011. Adaptability of birch (*Betula pendula* Roth) and aspen (*Populus tremula* L.) genotypes to different soil moisture conditions. *Forest Ecology and Management* **262**:1387–1399.
- Prioul JL, Chartier P. 1977. Partitioning of transfer and carboxylation components of intracellular resistance to photosynthetic CO₂ fixation: a critical analysis of the methods used. *Annals of Botany* **41**:789–800.
- Räisänen J, Hansson U, Ullerstig A, Döscher R, Graham LP, Jones C, Meier HEM, Samuelsson P, Willén U. 2004. European climate in the late twenty-first century: regional simulations with two driving global models and two forcing scenarios. *Climate Dynamics* **22**:13–31.
- Rezaei Nejad A, Van Meeteren U. 2008. Dynamics of adaptation of stomatal behaviour to moderate or high relative air humidity in *Tradescantia virginiana*. *Journal of Experimental Botany* **59**:289–301.
- Rezaei Nejad A, Harbinson J, Van Meeteren U. 2006. Dynamics of spatial heterogeneity of stomatal closure in *Tradescantia virginiana* altered by growth at high relative air humidity. *Journal of Experimental Botany* **57**:3669–3678.
- Sapeta H, Costa JM, Lourenço T, Maroco J, van der Linde P, Oliveira MM. 2013. Drought stress response in *Jatropha curcas*: growth and physiology. *Environmental and Experimental Botany* **85**:76–84.
- Sellin A. 2001. Hydraulic and stomatal adjustment of Norway spruce trees to environmental stress. *Tree Physiology* **21**:879–888.
- Sellin A, Lubenets K. 2010. Variation of transpiration within a canopy of silver birch: effect of canopy position and daily versus nightly water loss. *Ecohydrology* **3**:467–477.
- Sellin A, Tullus A, Niglas A, Öunapuu E, Karusion A, Löhmus K. 2013. Humidity-driven changes in growth rate, photosynthetic capacity, hydraulic properties and other functional traits in silver birch (*Betula pendula*). *Ecological Research* **28**:523–535.
- Sellin A, Niglas A, Öunapuu-Pikas E, Kupper P. 2014. Rapid and long-term effects of water deficit on gas exchange and hydraulic conductance of silver birch trees grown under varying atmospheric humidity. *BMC Plant Biology* **14**:72.
- Sharkey TD. 1985. Photosynthesis in intact leaves of C3 plants: physics, physiology and rate limitations. *Botanical Review* **51**:53–105.
- Talbott LD, Rahveh E, Zeiger E. 2003. Relative humidity is a key factor in the acclimation of the stomatal response to CO₂. *Journal of Experimental Botany* **54**:2141–2147.
- Tissue DT, Griffin KL, Turnbull MH, Whitehead D. 2005. Stomatal and non-stomatal limitations to photosynthesis in four tree species in a temperate rainforest dominated by *Dacrydium cupressinum* in New Zealand. *Tree Physiology* **25**:447–456.
- Torre S, Fjeld T, Gislerød HR, Moe R. 2003. Leaf anatomy and stomatal morphology of greenhouse roses grown at moderate or high air humidity. *Journal of the American Society for Horticultural Science* **128**:598–602.
- Tullus A, Kupper P, Sellin A, Parts L, Söber J, Tullus T, Löhmus K, Söber A, Tullus H. 2012a. Climate change at Northern latitudes: rising atmospheric humidity decreases transpiration, N-uptake and growth rate of hybrid aspen. *PLoS ONE* **7**:e42648.
- Tullus A, Rytter L, Tullus T, Weih M, Tullus H. 2012b. Short-rotation forestry with hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) in Northern Europe. *Scandinavian Journal of Forest Research* **27**:10–29.
- van de Weg MJ, Meir P, Grace J, Ramos GD. 2012. Photosynthetic parameters, dark respiration and leaf traits in the canopy of a Peruvian tropical montane cloud forest. *Oecologia* **168**:23–34.
- von Caemmerer S, Farquhar GD. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**:376–387.