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An experimental facility for free air humidity manipulation (FAHM) can alter water flux through deciduous tree canopy

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ABSTRACT

A facility for free air humidity manipulation (FAHM) was established to investigate the effect of increased air humidity on trees' performance and their canopy functioning with respect to rising air humidity predicted for Northern Europe. The FAHM system enables air relative humidity (RH) to be increased up to 18 units (%) over the ambient level during mist fumigation, depending on the wind speed inside the experimental stand. Water was dispersed inside 14 × 14 m experimental plots in the form of mist with an average particle size of 50 μm from June to August in 2008, and from May to September in 2009. The average increase in RH was 7 units (%) over the whole period of humidification in 2008 ($P < 0.05$). The average diurnal stem sap flux density per unit projected leaf area (F) in silver birch (*Betula pendula* Roth.) trees was 24.8% ($P < 0.05$) and 27.2% ($P < 0.01$) higher in control (C) plots compared to humidification (H) plots during misting in 2008 and 2009, respectively. However, the difference between C and H plots was statistically insignificant ($P > 0.05$) in silver birch on the days without misting. In hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) the average difference in F between C and H plots was 61.1% ($P < 0.001$) during mist fumigation in the summer of 2009. Nevertheless, the difference was considerable (38.8%; $P < 0.001$) also on the days without misting, reflecting the impact of plant inner factors on F as a result of long-term acclimation to fumigation. The leaves of silver birch in a humidified plot demonstrated up to 2.4 °C lower ($P < 0.05$) leaf temperature (T_L) compared to the control plot in 2009. The decline in T_L decreased the humidity gradient between leaf and air by about 1/3, whereas 2/3 of the effect was caused directly by changes in air humidity in the leaf boundary layer. Our preliminary data suggest that the FAHM experimental facility enables water fluxes through a deciduous tree canopy to be reduced and this effect is attributable both to the increased air humidity and decreased leaf temperature. Changes in these two basic factors may create considerable differences in the physiology, anatomy and nutrition of a whole tree, also affecting forest functioning in the light of global climate change.

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1. Introduction

Several experimental systems have been developed to estimate the effect of global warming (Nijs et al., 1996; Luxmoore et al., 1998) and increasing atmospheric carbon dioxide concentration on the functioning of trees and forest ecosystems (Norby et al., 1997; Hendrey et al., 1999; Kellomäki et al., 2000; Pepin and Körner, 2002). Although the impact of rising temperature and carbon dioxide concentration on woody plants is already well documented (Saxe et al., 1998, 2001; Karnosky et al., 2003; Ainsworth and Long, 2005), the influence of co-occurring changes in atmospheric humidity (induced by rain, mist and fog events) on an ecosystem level is almost unknown.

Patterns of air humidity and rainfall remain poorly understood for many regions of the world, especially in the context of climate change. Little is known about the effects of precipitation amount, frequency and intensity on ecosystem functioning (New et al., 2001; Gerten et al., 2008). Scenarios on future climate change predict wetter conditions at higher latitudes, and the amount of precipitation in Northern Europe will also probably increase (IPCC, 2007). Climate change scenarios for the year 2100 predict an increase in air temperature (by 2.3–4.5 °C) and precipitation (by 5–30%) in the Baltic region as well (Kont et al., 2003). Because the increased amount of precipitation is generally manifested through increased cloud cover and frequency of wet days, the air humidity will rise too. Moreover, as the global climate warms in response to an increase in greenhouse gases such as carbon dioxide and methane, the overall atmospheric content of water vapour will also increase because of the increasing water vapour capacity of the air.

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The driving force for evaporation from the canopy is the difference in water vapour pressure (VPD) or humidity gradient between leaf interior and surrounding atmosphere, which depends on air relative humidity (RH), leaf temperature (T_L) and air temperature (T_A). The increase in RH reduces VPD, and a simultaneous decrease in T_L brings about an additional reduction in VPD in the case of wet leaves (due to evaporation from the leaf surface). The decrease in VPD due to leaf wetting is very common in conditions of cloud forests, when the foliage of trees is cloud-immersed (Reinhardt and Smith, 2008; Johnson and Smith, 2008), or in crop fields irrigated by misting techniques or sprinklers (Grange and Hand, 1987; Caverio et al., 2009), but also in regions of frequent fog (Burgess and Dawson, 2004). Generally, a decrease in VPD leads to a decreased steady-state leaf transpiration rate in a wide range of tree species from different habitats (Pataki et al., 1998; Meinzer, 2003; Bovard et al., 2005; Hölscher et al., 2005). Nevertheless, decreasing VPD may also lead to increased stomatal conductance and to a consequent rise in transpiration rate in different plant species grown at high RH (Popířilová, 1996; Fordham et al., 2001; Nejad and van Meeteren, 2005). As both water vapour and carbon dioxide are important regulators of stomatal behaviour, it is essential to understand the stomatal control over these fluxes; without this, it is impossible to make reliable predictions concerning the responses of trees and even less, of ecosystems, to climate change.

Seedlings of two fast-growing deciduous tree species – silver birch and hybrid aspen – were planted to create an experimental forest ecosystem at the free air humidity manipulation facility (FAHM) site. Silver birch is a pioneer tree species widely distributed in boreal forests growing on mineral soils both in the maritime and the continental regions of Europe and Asia. The forecast impact of elevated temperature and atmospheric carbon dioxide concentration on photosynthesis, growth and production of allelochemicals are well documented in silver birch (Kellomäki and Wang, 2001; Kuokkanen et al., 2003; Riikonen et al., 2005) and offers good possibilities for detailed future research. Hybrid aspen, however, is the fastest-growing deciduous species in boreal conditions and is often used as a most suitable species for short-rotation plantation forestry in Northern Europe (Weih, 2004; Tullus et al., 2009). Since a global temperature increase could be more beneficial for deciduous tree species than for coniferous ones, the overall ecological and economic importance of deciduous species will probably grow at higher latitudes. However, the additional impact of possible changes in atmosphere humidity on their performance and growth needs further study.

Our free air humidity manipulation facility (<http://www.lote.ut.ee/FAHM/in-english>) is designed to alter water flux through the canopy during the formation of a deciduous forest stand. We use an integrated approach combining two different technologies to increase air humidity in experimental plots: a misting technique to atomize water and a FACE-like technology (Hendrey et al., 1999) to mix the produced mist with air inside the plots. Although misting is a widely used technique to reduce transpiration and canopy temperature in many horticultural crops (Grange and Hand, 1987), this is the first attempt to use this technique to study the functioning of trees and forest ecosystems in response to climate change. The experimental design of the FAHM facility enables the responses of trees to increased air humidity and concurrent changes in soil moisture, leaf wetness and temperature to be studied. Simultaneously with the aboveground study, attention will be paid to rhizosphere processes, and to the biological diversity of the understory and soil biota. The latter is important since ecosystem response to climate change depends on species diversity (Bengtsson et al., 2000), although the experimental evidence until now has been controversial (Niklaus et al., 2007).

Because changes in air humidity are always coupled with changes in leaf temperature (mediated through transpiration from

leaf surface), it is very complicated to manipulate these two variables independently in free-air conditions. Therefore, the findings of the FAHM experiment might usefully complete ecosystem warming experiments, which sometimes circumvent the effect of humidity gradient between leaf and air (primarily that resulting from temperature differences) on plants functioning.

In this paper, we present a description of the facility, design of the FAHM experiment and our preliminary results to demonstrate that the xylem sap flux density of trees is affected by artificial humidification in free-air conditions. We set up the hypothesis that xylem sap flow of fast-growing deciduous tree species is significantly reduced during mist fumigation, whereas the presumed increase in the stomatal opening cannot compensate for the decline in water flux through the canopy due to the effect of elevated RH.

2. Materials and methods

2.1. Description of study site and experimental plots

The experimental facility is located at Rõka village, Järvselja Experimental Forest District (58°24'N, 27°29'E, altitude 40–48 m), in south-eastern Estonia. The long-term average annual precipitation of the region is 650 mm and the average temperature is 17.0 °C in July and –6.7 °C in January. The growing season usually lasts 175–180 days from mid-April to October. The experimental area was established on an abandoned agricultural field in 2006–2007. The study site is a fenced area of 2.7 ha containing nine 14 m × 14 m experimental plots arranged in two rows and surrounded by a buffer zone. The relief of the site is gently sloping with a maximum difference in elevation of about 8 m. 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 11.4, and pH 5.7–6.3.

One-year-old seedlings of silver birch (*Betula pendula* Roth.) and micropropagated hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) plants were planted in the experimental area in spring (birch) and autumn (aspen) in 2006. The stand density in the buffer zone around the experimental plots is 2500 trees ha⁻¹; the density in the experimental plots is 10,000 trees ha⁻¹. In the spring of 2008, shortly before the experiment was started, the average height (±SE) of the trees in nine experimental plots varied from 102 ± 1.2 to 112 ± 1.8 cm for hybrid aspen and from 127 ± 4.7 to 163 ± 5.0 cm for silver birch.

To investigate the impact of soil biota and understory species diversity on ecosystem functioning, two different types of ground vegetation were established in the plots, representing either disturbed forest vegetation, such as that usually recorded in recent clear-cut areas in Järvselja forest, or early-successional vegetation, such as that in abandoned arable fields, with low diversity and a strong dominance of a few grass species. The experimental “forest community” was created by transplanting 0.5 × 0.5 × 0.2 m patches of forest understory vegetation from a nearby forest clear-cut, inserted in the centres of 1 m × 1 m frames between planted trees in the spring of 2006. Simultaneously, seeds of all forest herbaceous plant species fruiting in the autumn of 2006 were collected and the mixture of seeds was sown in the spaces between the transplants. Forest species successfully regenerated via vegetative or seed regeneration. Early successional species also emerging in “forest community” have been regularly weeded out until now. “Early successional community” was created by sowing *Phleum pratense* L.—a grass species naturally dominating in surrounding old fields. In addition, a few other early successional species emerged spontaneously.

The vegetation in each experimental plot is divided into quarters: north (silver birch in “forest community”), east (silver birch in “early successional community”), south (hybrid aspen in “forest

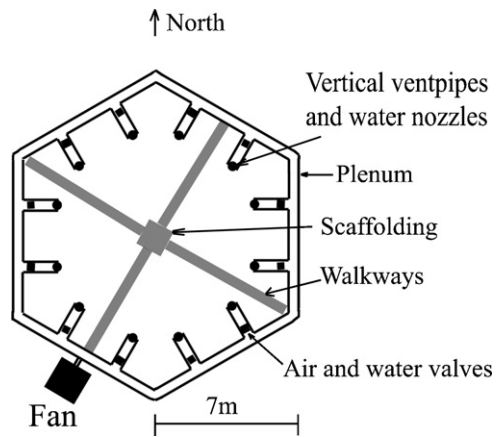


Fig. 1. A general layout of the FAHM experimental plot including main system hardware.

community”) and west (hybrid aspen in “early successional community”). Along the central line of each plot a thin weatherproof polycarbonate plastic plate (30 × 1400 × 0.8 cm; indicating height, length and width, respectively) was installed in the topsoil, to isolate the roots of two adjacent tree species. As the central line of the plots follows the natural slope of the site, the plate is not an obstacle for the horizontal flow of soil water.

2.2. Description of the FAHM system

The FAHM system hardware (Fig. 1) consists of a high-volume blower ($4\text{ m}^3\text{ s}^{-1}$, 11 kW), a plenum pipe (diameter 30 cm) for air distribution, 12 extendable vertical vent pipes (VVP; presently 3 m high) for emitting air, mist emitting nozzles, electrically operated valves at each VVP for turning on/off air and water flow, and a computer system to operate valves and to communicate with the central server. A centrifugal pump (1.2 kW) takes water from a nearby pond; water is filtered through a $20\text{ }\mu\text{m}$ replaceable filter and softened with a DME demineralizer (Prominent Dosiertechnik GmbH, Heidelberg, Germany). Five button-type misting nozzles (0.5 mm; Mist Cooling Inc., Richmond, TX) at each VVP, located vertically 30 cm apart from each other, atomize 4 l water per hour under 0.8 MPa pressure with a mist drop size of $\sim 50\text{ }\mu\text{m}$. Six computer-operated air and water valves are opened at the same time on the windward side. Air, coming out of slots cut into VVP-s, is used to help mix the mist emitted from nozzles more effectively.

Experimental plots with FAHM system hardware also have boxes with built-in Rabbit BL2600 microcomputer systems (Rabbit, Davis, CA). Each BL2600 has inputs connected to wind sensors (Windsonic; Gill Instruments, Lymington, UK), air temperature (T_A) and relative humidity (RH) sensors (HMP45A; Vaisala, Helsinki, Finland), and outputs connected to the air and water valves, water pump and blowers. The BL2600s are connected together with WIFI data-links to the central PC inside a small log-house at the site. Server/client software, written in Delphi 2007, allows the monitoring of wind speed and direction, air temperature and relative humidity inside and outside the experimental plots. The sensors are located in the centre of the plot 1.5 m above the ground and 10–15 m outside the plot as a reference. The operator can set up the program response time to wind changes. Data collected every 3–10 s are saved in a MS Access database.

The shortest distance between the neighbouring experimental plots is 26 m to minimize the drift of water vapour between the plots. In three experimental plots (H1, H2, H4) the air humidity was increased over the ambient level by fumigating trees with mist in the years 2008 and 2009. Fumigation was applied daily (Sundays excluded) from 9.00 to 17.00 h from the 1st of June to the 31st of

August in 2008; and from 9.00 to 19.00 h from the 5th of May to the 25th of September in 2009. Misting was carried out if the ambient RH was $<75\%$ and wind speed $<4\text{ m s}^{-1}$. The overall sum of misting was 253 h and 684 h in 2008 and 2009, respectively.

Five experimental plots (H3, C1, C2, C3 and C4) were used as control areas without misting in 2008. Although the sample plot H3 was provided with a high-volume blower like other H plots, mist was not added in this particular plot. Therefore, we used H3 plot as an additional (with blowing) control for the humidification treatment in 2008. Sample plots C1–C4 were not provided with a blower but served also as controls. In 2009 only the sample plots C1, C2 and C4 were used as controls, whereas the plots H3, C3 and D1 were surrounded with a 3 m high transparent film wall and served henceforth as “open-top” plots (not included in the current analysis). The experimental plot D1 is a prototype plot provided with an industrial air-dryer (RZ-101, Seibu Giken DST AB, Spanga, Sweden) to decrease air humidity below the ambient level.

2.3. Basic measurements in experimental plots

An automatic weather station (Campbell Scientific, Logan, UK) measuring wind speed and direction, photosynthetically active radiation, net solar radiation, air temperature, air relative humidity, precipitation and barometric pressure, has been collecting data on the site since November 2006. Several environmental variables were continuously measured and recorded in each experimental plot: air temperature and relative humidity (HMP45A humidity and temperature probe) in 3–4 replications at the mean height of the canopy; photosynthetically active radiation (LI-190SZ quantum sensor; LI-COR Biosciences, Lincoln, NE) in 3 replications at the mean height of the canopy; precipitation (TR-4 tipping bucket rain gauge; Texas Electronics, Dallas, TX) in 9 replications above the ground vegetation; soil temperature (ST1 soil temperature probe; Delta-T Devices, Burwell, UK) and soil water potential (EQ2 equitensiometer; Delta-T Devices) in 6 replications at depths of 15 and 30 cm. The readings of the sensors were collected every 10–60 s and stored as average values every 1–10 min with a data logger (DL2e; Delta-T Devices).

To estimate the amount of percolated soil water, plate lysimeters made from stainless steel with a collecting area of 627 cm^2 were installed in the soil at a depth of 40 cm in June 2008. Polyethylene tubes connected the lysimeters with water collectors (5000 ml polyethylene canisters disposed at a depth of 1 m). Water from the canisters was sampled, using a peristaltic vacuum pump, through a plastic pipe. Each quarter of the experimental plot contains one lysimeter. For rhizosphere studies, the fine root (diameter $<2\text{ mm}$) turnover in the sample plots will be estimated by the minirhizotron technique. In each quarter of a plot, three transparent acrylic tubes with an inner diameter of 50 mm were installed in the soil at a 45° angle.

2.4. Measurements of plant variables

The xylem sap flow of sample trees was recorded with five sap flow systems of T4.2 (EMS Brno, Brno, Czech Republic) provided with ‘Baby Kučera’ sap flow sensors for 8–12 and 12–18 mm stem diameter in 2008. Six sap flow systems FLOW4 (Dynamax Inc., Houston, TX) coupled with STG 25 stem gauges were used in addition to the T4.2 systems in 2009. Four silver birch trees from the centre of each plot were fitted with sap flow gauges from July to October in 2008. In 2009, four sample trees of both silver birch and hybrid aspen from the centre of each experimental plot were fitted with sap flow gauges from May to September. In both years half of the sample trees were selected from the “forest community” and the other half from the “early successional community”. The sap flow data was recorded every 1 min and stored as 10-min aver-

ages by T4.2 and as 30-min averages by FLOW4. For 'Baby Kučera' sensors the average values recorded in humid nights (RH > 95%) were used in baseline subtraction procedure in both years. Baseline values were calculated for every 5-day interval over the whole measurement period to exclude the effect of increased stem heat storage capacity on the determination of the baseline value.

To express the sap flux density per unit projected leaf area (F ; $\text{mmol m}^{-2} \text{s}^{-1}$), the total leaf area was determined at the end of July in 2008 and at the beginning of August in 2009. For that purpose, all the leaves of sample trees were counted and 20–50 sample leaves were randomly collected and their area measured with a laser area meter CI-203 (CID Inc., Camas, WA) or with an optical area meter LI-3100C (LI-COR Biosciences, Lincoln, NE). The total leaf area was calculated from the area of sampled leaves and the total number of leaves.

Leaf temperature (T_L) was measured on two sample trees of silver birch in plots C1 and H1 using leaf temperature probes (MT2; Delta-T Devices) in 2009. Each of the four trees was provided with 3 sensors located in the lower, middle and upper thirds of the canopy. Additionally, the temperature of leaves was measured episodically with a thermal camera Thermacam FC 640 (FLIR Systems AB, Danderyd, Sweden).

2.5. Data analysis

For comparison of the experimental data between the control (C plots) and misting treatment (H plots), a repeated measure analysis of variance (ANOVA) was applied on datasets. Repeated datasets of three summer months (from June to August) were used to compare the averages of RH, air temperature, soil water potential and precipitation in 2008. The daily averages of sap flux density during mist fumigation were analysed in July and August 2008 (on 34 days) and in July 2009 (on 8 days). The data sets of non-fumigated days were based on the measurements of 9 consecutive Sundays in July and August 2008 (from 9:00 to 17:00 h), and on 3 Sundays in July 2009 (from 9:00 to 19:00 h). The number of analysed days depended on the comparability of the datasets between individual plots: only the days without any gaps in sap flow data were included into the analysis.

To find out differences in leaf temperature, leaf-to-atmosphere humidity gradient and canopy conductance, the daily averages during mist fumigation were analysed with repeated ANOVA on 11 days in July and August of 2009. The canopy conductance to water

vapour (g_c) was calculated according to Fick's law:

$$g_c = F / (w_i - w_a) \quad (1)$$

where F is sap flux density; w_i and w_a are the mole fractions of water vapour in leaf intercellular spaces and in the atmosphere, respectively. To derive g_c from F , we assumed the capacitance of small stems to be negligible. Moreover, we used only the daily averages of F in the data analysis, which minimises the possible stem capacitance effect (observable on a diurnal scale) on canopy conductance.

The plate lysimeter data were analysed with a Student's t -test and the relationship between RH change and wind speed inside the misting plots was described using nonlinear regression analysis. Data analysis was carried out using the computer package Statistica, Version 6.0 (StatSoft Inc., Tulsa, OK).

3. Results

3.1. Effect of humidification on environmental variables

Our results suggest that the free air humidification facility enables air humidity to increase considerably over the ambient level inside a young deciduous forest stand. The misting resulted in about 7 units (%) higher RH in humidification plots compared with the control plots ($P < 0.05$) during the mist fumigation applied from June to August in 2008 (Fig. 2A). Although the air temperature (T_A) varied among the experimental plots, the difference between the treatments was negligible (Fig. 2B). The increase and variability of air humidity depended mainly on wind velocity (u) inside the experimental stand. With increasing u the mist was carried quickly through the plot and the efficiency of air humidification decreased (Fig. 3). However, on calm days the increase in RH reached up to 18% inside plot H1, which was characterised by the lowest u .

The mist fumigation did not increase the amount of precipitation in H plots (Fig. 4A). Although soil water potential (Ψ_S) demonstrated lower values in the control plots compared to H plots from June to August (Fig. 4B), the difference was statistically insignificant. However, when Ψ_S was analysed by single months the difference turned out to be significant in August ($P < 0.05$). The impact of different tree species on percolation water collected with plate lysimeters was significant for H plots ($P < 0.05$), but not for C plots. The effect of misting was more pronounced for hybrid aspen: the leachate formed 0.98 and 0.07 mm day^{-1} in H and C treatments,

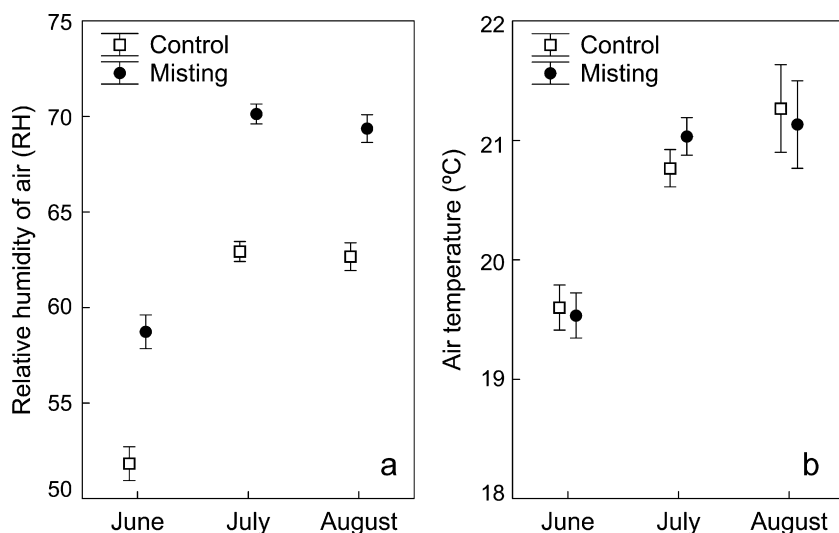


Fig. 2. Monthly average values of air relative humidity (A) and air temperature (B) in control ($n = 3$) and misting ($n = 3$) plots during mist fumigation from June to August in 2008. The bars denote standard error of the mean.

Table 1

Daily average values of stem sap flux density ($\text{mmol m}^{-2} \text{s}^{-1}$) of sample trees in control ($n=5$ in 2008; $n=3$ in 2009) and misting ($n=3$) plots during mist fumigation and on misting-free days in the summers of 2008 and 2009.

Year	Tree species	During mist fumigation			Without mist fumigation		
		C plots	H plots	P value	C plots	H plots	P value
2008	Silver birch	1.41	1.06	<0.05	1.04	0.98	ns
2009	Silver birch	1.03	0.75	<0.01	0.96	1.01	ns
2009	Hybrid aspen	1.26	0.49	<0.001	1.03	0.63	<0.001

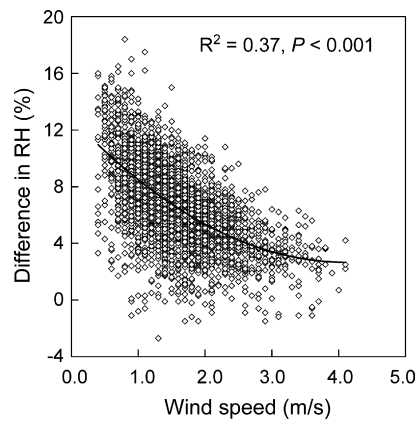


Fig. 3. Relationship between wind speed and the difference in air relative humidity (RH) inside and outside the experimental plot H1 in July 2008.

respectively ($P < 0.001$); for silver birch the respective values were 0.54 and 0.04 mm day^{-1} ($P < 0.05$).

3.2. Effect of mist fumigation on tree and leaf variables

In silver birch, the mean sap flux density (F) per unit leaf area was 24.8% ($P < 0.05$) higher in C plots compared to H plots during mist fumigation from July to August in 2008 (Fig. 5; Table 1). However, on the days without fumigation (Sundays), F did not differ significantly between misting and control (Fig. 6; Table 1). F of sample trees was significantly lower in misting plots than in control ones during mist fumigation also in July 2009; the average difference between H and C plots was 27.2% in silver birch and 61.1% in hybrid aspen. Surprisingly, F in hybrid aspen was 38.8% ($P < 0.001$) smaller in H plots even on Sundays when misting was not carried out (Table 1).

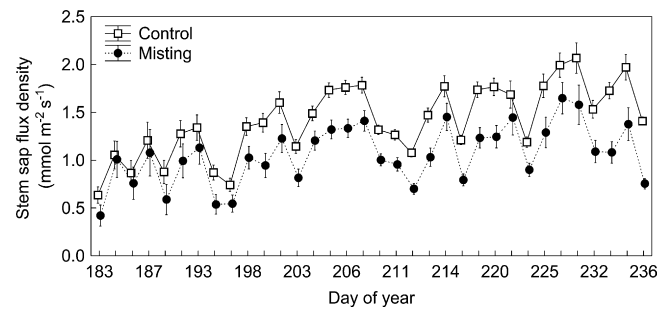


Fig. 5. Daily average values of stem sap flux density in silver birch trees during mist fumigation in control ($n=5$) and misting ($n=3$) plots from July to August in 2008. The bars denote standard error of the mean.

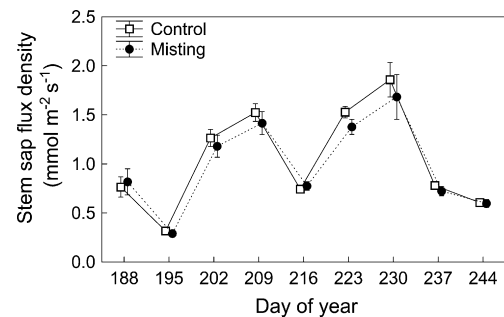


Fig. 6. Daily average values of stem sap flux density in silver birch trees on misting-free days in control ($n=5$) and misting ($n=3$) plots from July to August in 2008. The bars denote standard error of the mean.

The leaves of silver birch demonstrated on average 2.4°C lower temperature in H1 plot compared to C1 plot ($P < 0.05$; Table 2). The humidity gradient (ΔN_{wv}) between leaf and atmosphere was 38% lower in the trees of the H1 plot during mist fumigation. How-

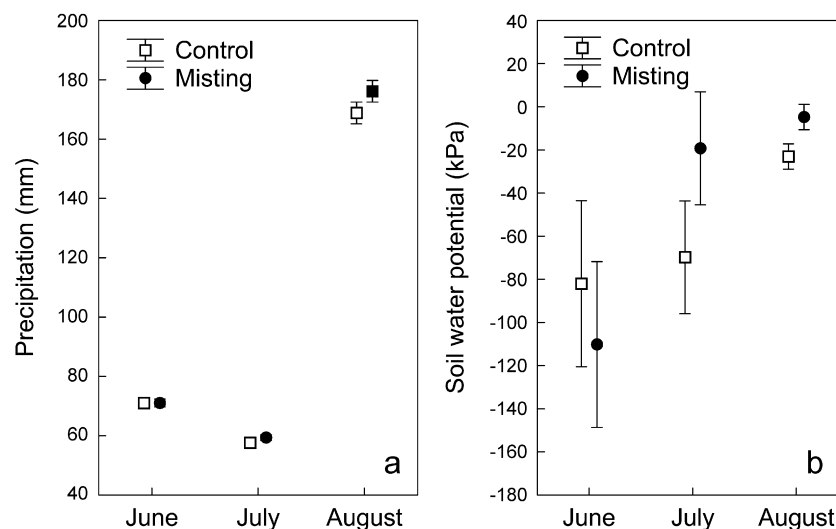


Fig. 4. Monthly sum of precipitation (A) and monthly average values of soil water potential (B) in control ($n=3$) and misting ($n=3$) plots from June to August in 2008. The bars denote standard error of the mean.

Table 2
Average values of leaf temperature, humidity gradient between leaf and atmosphere, sap flux density, and canopy conductance in silver birch trees in control plot C1 ($n=2$) and misting plot H1 ($n=2$) during mist fumigation in July and August 2009.

Parameter	C1 plot	H1 plot	P value	Difference (%)
Leaf temperature ($^{\circ}\text{C}$)	22.3	19.9	<0.05	11
Humidity gradient (mmol mol^{-1})	9.29	5.71	<0.05	38
Theoretical humidity gradient ^a (mmol mol^{-1})	9.29	7.02	<0.05	24
Sap flux density ($\text{mmol m}^{-2} \text{s}^{-1}$)	1.1	0.76	ns	31
Canopy conductance ($\text{mmol m}^{-2} \text{s}^{-1}$)	127	137	ns	8

^a Theoretical humidity gradient assumes that the leaf temperatures in H and C plots are equal.

ever, when we calculated a theoretical humidity gradient, which excludes the effect of leaf temperature on differences in ΔN_{wv} between the treatments (assuming that the average leaf temperature in H1 plot was as high as in C1 plot), then the difference was 24% (Table 2). Thus, about 1/3 of the difference in leaf-to-air humidity gradient between control and misting treatment was attributable to the lowered leaf temperature, and about 2/3 to the increased air humidity during mist fumigation. At the same time, the sap flux density of the sample trees in the H1 plot decreased 31% compared to the trees in the control plot (Table 2). However, the difference turned out to be statistically insignificant ($P>0.05$), probably because of high variation among the trees and the limited number of sample trees. Although canopy conductance was slightly (by 8%) higher for the trees in the H1 plot during mist fumigation, the difference was not significant between the treatments ($P>0.05$).

4. Discussion

Although several experimental studies have demonstrated the impact of increased air humidity on plant water relations and gas exchange (Fordham et al., 2001; Leuschner, 2002; Nejad and van Meeteren, 2005; Cunningham, 2006), there is still not much information on how increased air humidity could affect trees and forest functioning in the light of global climate change. Several investigations have been focused on rather short-term effects of high VPD or misting on flowers (Gilsleröd and Mortensen, 1990; Torre et al., 2003), field crops (Nijs et al., 1996; del Amor and Marcelis, 2005; Cavero et al., 2009) and herbaceous species grown in growth chambers (Leuschner, 2002; Nejad and van Meeteren, 2005). Although recent studies have focused on the effect of VPD in woody species, knowledge of long-term impacts of increased humidity and leaf wetting on trees and forest functioning has been scant and needs further studies (Cunningham, 2006; Dietz et al., 2007; Lenzion and Leuschner, 2008).

Our results suggest that mist fumigation in free-air conditions enables humidity gradient between leaf and atmosphere to be decreased by up to 38%, whereas about 1/3 of this impact could be explained by the decrease in leaf temperature. This is due to evaporation from the wet leaf surface, which is a common phenomenon in cloud or redwood forests (Burgess and Dawson, 2004). Even 6.6 $^{\circ}\text{C}$ lower leaf temperature in *Rhododendron catawbiense* and *Abies fraseri* seedlings under cloud immersed conditions (Johnson and Smith, 2008) has been observed. Such differences in T_L may create significant changes in many physiological processes in a plant leaf, including photoinhibition (Johnson and Smith, 2008) dark respiration (Bunce, 2007) and changes in phenology (Vitasse et al., 2009).

However, despite the lower T_L during mist fumigation, the major component determining humidity gradient between leaf and atmosphere was the air humidity. In general, the humidity treatment (including the effects of air humidity, leaf temperature and leaf wetting) decreased sap flux density during mist fumigation more than 20–25%. The difference in humidity-induced changes of F between silver birch and hybrid aspen trees suggests that these fast-growing

species behave differently under misting stress, at least at a young age. Nevertheless, it is likely that the differences observed in F in hybrid aspen trees between the treatments (i.e. large difference on misting and substantial difference on misting-free days) result also from plant intrinsic traits, reflecting long-term acclimation to mist fumigation.

A long-term reduction in sap flux density or transpiration rate of such a magnitude should affect both tree and ecosystem functioning, because water fluxes are closely related to carbon and nutrient fluxes. Several studies (McDonald et al., 2002; Cramer et al., 2009) have shown that decreased transpiration may restrict mass flow of mineral nutrients from soil and roots up to the foliage. A high rate of nutrient acquisition seems to be especially important in fast-growing and shade-intolerant tree species that are characterised by high leaf nitrogen content and considerable night-time stomatal conductance and transpiration (Daley and Phillips, 2006; Marks and Lechowicz, 2007). Intense night-time transpiration has been observed in natural and climate chamber conditions also in silver birch and hybrid aspen (Sellin and Lubenets, in press; P. Kupper, unpublished data). It could be that a considerable decrease in tree canopy transpiration may lead to decreased growth and productivity in fast-growing tree species like silver birch and hybrid aspen if humidity and precipitation increase as a result of future climate trends; this could be more pronounced in Northern regions where soil water availability usually does not limit tree growth.

The decrease in sap flow or transpiration rate as a consequence of increasing RH is a universal response in various tree species. However, in some cases the decreasing VPD may lead to increased stomatal conductance and a consequent increase in transpiration rate (Popšílová, 1996; Fordham et al., 2001; Nejad and van Meeteren, 2005). Nevertheless, this was not the case in our study, because the canopy conductance was not significantly higher in the misting plot compared to the control plot (Table 2). It is probable that the slightly higher (statistically insignificant) canopy conductance recorded in trees of the H plot was due to higher soil water availability. This idea is supported by the data of plate lysimeters, which collected a much higher amount of leached water in misting plots than in control ones. Also, the soil water potential was higher in the misting treatment, although it was statistically significant only in August. In general, the Ψ_s varied highly within treatments, and this could be one reason why it was not significantly higher in the misting treatment in June and July 2008. The lysimetric data showed clear differences in the amount of percolated water also between birch and aspen stands within the experimental plots. This was probably because of overall higher size and leaf area of silver birch trees, which led to higher stand evapotranspiration rate.

The larger amount of percolated water and also higher Ψ_s in H plots in August 2008 might be the result of the decreased transpiration of trees and ground vegetation caused by the increased RH, as well as the irrigating effect of misting in the form of occult precipitation. Soil water saving effect has been demonstrated in response to decreased stomatal conductance and transpiration rate in several tree species growing under elevated carbon dioxide concentration (Wullschleger et al., 2002; Leuzinger and Körner, 2007).

To summarise, our results suggest that the FAHM system allows water fluxes through tree canopies to be altered and, as a consequence, tree (and likely ecosystem) functioning in field conditions to be affected. However, the effect of misting on sap flow and other physiological processes is probably quite variable in different summers because of the natural variability of air humidity, solar radiation, and rainfall from year to year. Nevertheless, the lower daytime sap flux density and leaf temperature during mist fumigation may induce considerable changes in leaf anatomical traits, tree respiration rate, photosynthesis, stomatal behaviour, water-use efficiency, biomass allocation, growth, and phenology in fast-growing tree species like silver birch and hybrid aspen.

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References

- Ainsworth, E.A., Long, S.P., 2005. What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytologist* 165, 351–372.
- del Amor, F.M., Marcellis, L.F.M., 2005. Regulation of growth and nutrient uptake under different transpiration regimes. *Acta Horticulturae* 697, 523–528.
- Bengtsson, J., Nilsson, S.G., Franc, A., Menozzi, P., 2000. Biodiversity, disturbances, ecosystem function and management of European forests. *Forest Ecology and Management* 132, 39–50.
- Bovard, B.D., Curtis, P.S., Vogel, C.S., Su, H.-B., Schmid, H.P., 2005. Environmental controls on sap flow in a northern hardwood forest. *Tree Physiology* 25, 31–38.
- Bunce, J.A., 2007. Direct and acclamatory responses of dark respiration and translocation to temperature. *Annals of Botany* 100, 67–73.
- Burgess, S.S.O., Dawson, T.E., 2004. The contribution of fog to the water relations of *Sequoia sempervirens* (D. Don): foliar uptake and prevention of dehydration. *Plant, Cell and Environment* 27, 1023–1034.
- Cramer, M.D., Hawkins, H.-J., Verboom, G.A., 2009. The importance of nutritional regulation of plant water flux. *Oecologia* 161, 15–24.
- Cunningham, S.C., 2006. Effects of vapour pressure deficit on growth of temperate and tropical evergreen rainforest trees of Australia. *Acta Oecologica* 30, 399–406.
- Daley, M.J., Phillips, N.G., 2006. Interspecific variation in nighttime transpiration and stomatal conductance in a mixed New England deciduous forest. *Tree Physiology* 26, 411–419.
- Dietz, J., Leuschner, C., Hölscher, D., Kreilein, H., 2007. Vertical patterns and duration of surface wetness in an old-growth tropical montane forest, Indonesia. *Flora* 202, 111–117.
- Fordham, M.C., Harrison-Murray, R.S., Knight, L., Evered, C.E., 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.
- Cavero, J., Medina, E.T., Puig, M., Martinez-Cob, A., 2009. Sprinkler irrigation changes maize canopy microclimate and crop water status, transpiration, and temperature. *Agronomy Journal* 101, 854–864.
- Gerten, D., Luo, Y., LeMarie, G., Parton, W.J., Keough, C., Weng, E., Beier, C., Ciais, P., Cramer, W., Dukes, J.S., Hanson, P.J., Knapp, A.A.K., Linder, S., Nepstad, D., Rustad, L., Sowerby, A., 2008. Modelled effects of precipitation on ecosystem carbon and water dynamics in different climatic zones. *Global Change Biology* 14, 2365–2379.
- Gilsleröd, H.R., Mortensen, L.M., 1990. Relative humidity and nutrient concentration affect nutrient uptake and growth of *Begonia × hiemalis*. *HortScience* 25, 524–526.
- Grange, R.L., Hand, D.W., 1987. A review of the effects of atmospheric humidity on the growth of horticultural crops. *Journal of Horticultural Science* 62, 125–134.
- Hendrey, G.R., Ellsworth, D.E., Lewin, K.F., Nagy, J., 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO₂. *Global Change Biology* 5, 293–309.
- 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 Structure and Function* 19, 628–637.
- IPCC, 2007. *Climate Change 2007: The Physical Science Basis*. Cambridge University Press, Cambridge.
- Johnson, D.M., Smith, W.K., 2008. Cloud immersion alters microclimate, photosynthesis and water relations in *Rhododendron catawbiense* and *Abies fraseri* seedlings in the southern Appalachian Mountains, USA. *Tree Physiology* 28, 385–392.
- Karnosky, D.F., Zak, D.R., Pregitzer, K.S., Awmack, C.S., Bockheim, J.G., Dickson, R.E., Hendrey, G.R., Host, G.E., King, J.S., Kopper, B.J., Kruger, E.L., Kubiske, M.E., Lindroth, R.L., Mattson, W.J., McDonald, E.P., Noormets, A., Oksanen, E., Parsons, W.F.J., Percy, K.E., Podila, G.K., Riemenschneider, D.E., Sharma, P., Thakur, R., Söber, A., Söber, J., Jones, W.J., Anttonen, S., Vapaavuori, E., Mankovska, B., Heilmann, W., Isebrands, J.G., 2003. Tropospheric CO₂ moderates responses of temperate hardwood forests to elevated CO₂: a synthesis of molecular to ecosystem results from the Aspen FACE project. *Functional Ecology* 17, 289–304.
- Kellomäki, S., Wang, K.-Y., Lemmetinen, M., 2000. Controlled environment chambers for investigating tree response to elevated CO₂ and temperature under boreal conditions. *Photosynthetica* 38, 69–81.
- Kellomäki, S., Wang, K.-Y., 2001. Growth and resource use of birch seedlings under elevated carbon dioxide and temperature. *Annales de Botany* 87, 669–682.
- Kont, A., Jaagus, J., Aunap, R., 2003. Climate change scenarios and the effect of sea-level rise for Estonia. *Global and Planetary Change* 36, 1–15.
- Kuokkanen, K., Yan, S.C., Niemelä, P., 2003. Effects of elevated CO₂ and temperature on the leaf chemistry of birch *Betula pendula* (Roth) and the feeding behaviour of the weevil *Phyllobius maculicornis*. *Agricultural and Forest Entomology* 5, 209–217.
- Lendzion, J., Leuschner, C., 2008. Growth of European beech (*Fagus sylvatica* L.) saplings is limited by elevated atmospheric vapour pressure deficits. *Forest Ecology and Management* 256, 648–655.
- Leuschner, C., 2002. Air humidity as an ecological factor for woodland herbs: leaf water status, nutrient uptake, leaf anatomy, and productivity of eight species grown at low or high vpd levels. *Flora* 197, 262–274.
- Leuzinger, S., Körner, C., 2007. Water savings in mature deciduous forest trees under elevated CO₂. *Global Change Biology* 13, 2498–2508.
- Luxmoore, R.J., Hanson, P.J., Beauchamp, J.J., Joslin, J.D., 1998. Passive nighttime warming facility for ecosystem research. *Tree Physiology* 18, 615–623.
- Marks, C.O., Lechowicz, M.J., 2007. The ecological and functional correlates of nocturnal transpiration. *Tree Physiology* 27, 577–584.
- McDonald, E.P., Erickson, J.E., Kruger, E.L., 2002. Can decreased transpiration limit plant nitrogen acquisition in elevated CO₂? *Functional Plant Biology* 29, 1115–1120.
- Meinzer, F.C., 2003. Functional convergence in plant responses to the environment. *Oecologia* 134, 1–11.
- Nejad, A.R., van Meeteren, U., 2005. Stomatal response characteristics of *Tradescantia virginiana* grown at high relative air humidity. *Physiologia Plantarum* 125, 324–332.
- New, M., Todd, M., Hulme, M., Jones, P., 2001. Precipitation measurements and trends in the twentieth century. *International Journal of Climatology* 21, 1899–1922.
- Nijs, I., Kockelbergh, F., Teughels, H., Blum, H., Hendrey, G., Impens, I., 1996. Free air temperature increase (FATI): a new tool to study global warming effects on plants in the field. *Plant, Cell and Environment* 19, 495–502.
- Niklaus, P.A., Alpehi, J., Kampichler, C., Kandeler, E., Körner, C., Tscherko, D., 2007. Interactive effects of plant species diversity and elevated CO₂ on soil biota and nutrient cycling. *Ecology* 88, 3153–3163.
- Norby, R.J., Edwards, N.T., Riggs, J.S., Abner, C.H., Wullschlegel, S.D., Gunderson, C.A., 1997. Temperature-controlled open-top chambers for global change research. *Global Change Biology* 3, 259–267.
- Pataki, D.E., 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.
- Pepin, S., Körner, C., 2002. Web-FACE: a new canopy free-air CO₂ enrichment system for tall trees in mature forests. *Oecologia* 133, 1–9.
- Popišilová, J., 1996. Effect of air humidity on the development of functional stomatal apparatus. *Biologia Plantarum* 38, 197–204.
- Reinhardt, K., Smith, W.K., 2008. Impacts of cloud immersion on microclimate, photosynthesis and water relations of *Abies fraseri* (Pursh.) Poiré in a temperate mountain cloud forest. *Oecologia* 158, 229–238.
- Riikonen, J., Holopainen, T., Oksanen, E., Vapaavuori, E., 2005. Leaf photosynthetic characteristics of silver birch during three years of exposure to elevated concentrations of CO₂ and O₃ in the field. *Tree Physiology* 25, 621–632.
- Saxe, H., Cannell, M.G.R., Johnsen, Ø., Ryan, M.G., Vourliitis, G., 2001. Tree and forest functioning in response to global warming. *New Phytologist* 149, 369–400.
- Saxe, H., Ellsworth, D.S., Health, J., 1998. Tree and forest functioning in an enriched CO₂ atmosphere. *New Phytologist* 139, 395–436.
- 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*, doi:10.1002/eco.133.
- Torre, S., Fjeld, T., Gilsleröd, H.R., 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., Tullus, H., Soo, T., Pärn, L., 2009. Above-ground biomass characteristics of young hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) plantations on former agricultural land in Estonia. *Biomass and Bioenergy* 33, 1617–1625.
- Vitasse, Y., Porté, A.J., Kremer, A., Michalet, R., Delzon, S., 2009. Responses of canopy duration to temperature changes in four temperate tree species: relative contributions of spring and autumn leaf phenology. *Oecologia* 161, 187–198.
- Weih, M., 2004. Intensive short rotation forestry in boreal climates: present and future perspectives. *Canadian Journal of Forest Research* 34, 1369–1378.
- Wullschlegel, S.D., Tschaplinski, T.J., Norby, R.J., 2002. Plant water relations at elevated CO₂—implication for water-limited environments. *Plant, Cell and Environment* 25, 319–331.