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Titanium dioxide (TiO2) fine particle capture and BVOC emissions of Betula pendula and Betula pubescens at different wind speeds

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Title: Titanium dioxide (TiO_2) fine particle capture and BVOC emissions of *Betula pendula* and *Betula pubescens* at different wind speeds

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Abstract

Trees are known to affect air quality by capturing a remarkable amount of particles from the atmosphere. However, the significance of trees in removing very fine particles (diameter less than 0.5 µm) is not well understood. We determined particle capture efficiency (C_p) of two birch species: *Betula pendula* and *Betula pubescens* by using inert titanium dioxide fine particles (TiO_2, geometric mean diameter 0.270 µm) at three wind speeds (1, 3 and 6 m s^{-1}) in a wind tunnel. Capture efficiencies were determined by measuring densities of TiO_2 particles on leaf surfaces by scanning electron microscopy. In addition, the particle intake into an inner structure of leaves was studied by transmission electron microscopy. The effects of fine particle exposure and wind speed on emission rates of biogenic volatile organic compounds (BVOCs) were measured. Particles were captured (C_p) equally efficiently on foliage of *B. pendula* (0.0026 ± 0.0005) % and *B. pubescens* (0.0025 ± 0.0006) %. Increasing wind speed significantly decreased C_p. Increasing wind speed increased deposition velocity (V_g) on *B. pendula* but not on *B. pubescens*. Particles were deposited more efficiently on the underside of *B. pendula* leaves, whereas deposition was similar on the upper and under sides of *B. pubescens* leaves. TiO_2 particles were found inside three of five *B. pendula* leaves exposed to particles at a wind speed of 1 m s^{-1} indicating that particles can penetrate into the plant structure. Emission rates of several mono-, homo- and sesquiterpenes were highest at a wind speed of 3 m s^{-1} in *B. pendula*. In *B. pubescens*, emission rates of a few monoterpenes and nonanal decreased linearly with wind speed, but emission rates of sesquiterpenes were lowest at 3 m s^{-1} and increased at 6 m s^{-1}. Emission rates of a few green leaf volatile compounds increased with increasing wind speed in both species. The results of this study suggest that the surface structure of trees is less important for capturing particles with a diameter of ca 0.3 µm than for larger particles. Airborne fine particles penetrated into the intercellular space of the leaf via stomata, and this

mechanism should be studied further for a better understanding of nanomaterial accumulation in nature. Wind can affect BVOC emissions and composition.

Keywords: Fine particles, BVOCs, particle intake, deposition, birch, wind speed

1. Introduction

Trees remove a considerable amount of particulate matter from the atmosphere by capturing it on foliage (Beckett et al. 2000). This particle removal capacity of trees is one of the ecosystem services that have been estimated to be worth millions of dollars (e.g. 60.1 million \$ y^{-1} in New York City, U.S.) in health care savings through improved air quality (Nowak et al. 2013). This estimation was made for particles in the size range of $PM_{2.5}$ (Nowak et al. 2013), which has been shown to be the most harmful to human health (Schwartz et al. 1996). More adverse health effects are usually related to particles in the lower range of the $PM_{2.5}$ (i.e. below 1 µm) (Oberdörster et al. 2005), but the removal capacity of these particles by trees or other vegetation is rarely studied (Lin and Khlystov 2012).

Particle capture on trees is a complicated process. When impaction is the main deposition method, physical characteristics of particles, such as a larger size and faster velocity, increase the deposition rate, but key parameters of foliage, such as a smaller leaf size, complex structure and reduced transpiration, also increase capture of particles with a size over 0.5 µm (Beckett et al. 2000; Räsänen et al. 2012; 2013). Thermal movement known as Brownian diffusion is the main

deposition method for particles smaller than 0.5 µm (Hinds 1999). In fact, deposition of ultrafine particles ($PM_{0.1}$, diameter below 0.1 µm) can increase with decreasing particle size (Grantz et al. 2003; Lin and Khlystov 2012). Leaf structural characteristics have an influence on particle deposition on surfaces. For example, the presence of abundant structural waxes has been shown to increase the particle load on leaf surfaces (Wedding et al. 1975; Burkhardt et al. 1995). Foliar particle capture efficiency (C_p) of particles with an average size of 0.7 µm was observed to be twice as high for hairy pubescent birch (*Betula pubescens*) than for less hairy silver birch (*Betula pendula*) (Räsänen et al. 2013). Very little is known about the penetration of solid particulates into plant leaves, but earlier studies have shown that hydrophilic nanoparticles (fluorescent polystyrene particles with carboxylate-modified surfaces: FluoSpheres, Trans-FluoSpheres) in water suspension can penetrate through the stomata of leek (*Allium porrum*) and broad bean (*Vicia faba*) (Eichert et al. 2008).

Trees also affect air quality by emitting biogenic volatile organic compounds (BVOCs) which plants need e.g. for reproduction and defense against abiotic and biotic stress factors (Holopainen 2011). Calfapietra et al. (2013) suggested that low BVOC-emitting tree species should be favored in the VOC-limited urban areas to reduce formation of ozone, an air pollutant with harmful effects on humans and vegetation. However, BVOCs break down ozone which can lead to new particle formation and cloud formation that could locally cool the climate (Kavouras et al. 1998; Holopainen 2011). Terpenoids, such as monoterpenes and sesquiterpenes, are particularly reactive BVOCs in the atmosphere (Atkinson and Arey 2003). Of the European tree species, *B. pendula* and *B. pubescens* have high emission potentials for sesquiterpenes and moderate emission potentials for monoterpenes (Steinbrecher 2009). Holopainen and Gershenzon (2010) summarized a number of external factors (e.g. elevated temperature, high light intensity, water and salt stress, herbivore

95 damage and pathogen infection) that lead to higher BVOC emissions from vegetation. Effects of
96 subtler external factors, such as wind speed or particle pollution, on BVOC emissions are not well
97 understood. Juuti et al. (1990) showed that pronounced needle movement caused by air movement
98 had no detectable effect on monoterpene emissions of Monterey pine (*Pinus radiata*). The results of
99 a more recent study suggested that wind induced abrasion of branches increased monoterpene
100 emissions from *Chamaecyparis obtusa* (Japanese cypress) (Mochizuki et al. 2011). Birch leaves
101 contain glandular trichomes that are storage structures for volatile terpenoids (Biswas et al. 2009).
102 Glandular trichomes are a likely source of sesquiterpenes in *Betula* species, since their density was
103 shown to correlate positively with sesquiterpene emission in *B. nana* leaves (Schollert et al. in
104 press). Wind can also cause membrane damage and induce emissions of lipoxygenase products,
105 such as many green leaf volatiles (GLVs) (Creelman and Mullet 1997, Vuorinen et al. 2004, Scala
106 et al. 2013). Thus, in birches, the subtle mechanical damage by wind or particles on leaf surfaces
107 might increase BVOC emissions. In nature, wind can modulate plant and leaf properties that affect
108 particle capture by foliage. Wind influences tree growth and function by regulating factors such as
109 plant structure and posture, gas exchange, heat transfer, water use efficiency, abrasion and visible
110 damage (Grace 1988; Knight et al. 1992; de Langre 2008). A higher wind speed can lower stomatal
111 conductance (Räsänen et al. 2012), which can control emissions of water-soluble BVOCs
112 (Niinemets et al. 2004; Harley et al. 2014) and potentially affect the intake of particles.

113

114 The aim of this study was to determine how efficient silver birch (*Betula pendula*) and pubescent
115 birch (B*etula pubescens*) are at capturing dry and inert titanium dioxide (TiO_2) fine particles. We
116 also wanted to find out if particles can penetrate through the stomata into the intercellular space of
117 birch leaves. Other aims were to reveal if increasing wind speed and inert fine particle deposition
118 can change the BVOC emissions of birches.

2. Material and methods

2.1. Wind tunnel and particle exposure devices

The wind tunnel system (described in detail by Räsänen et al. 2012) consisted of a six meter long air duct with a diameter of 50 cm. Saplings were placed one at a time into a transparent section of the tunnel that was illuminated with a greenhouse lamp at a photosynthetically active radiation (PAR) level of 450 µmol m^{-2} s^{-1} during a two-hour run (Räsänen et al. 2013). Artificial fine particles with a geometric mean diameter (GMD) of 270 nm and a geometric standard deviation (GSD) of 1.6 were produced from TiO_2 powder (Inframat®, Manchester, CT, USA) with a powder disperser (Palas RGB 1000, Palas, Germany). TiO_2 fine particles have two main different forms referred to as 'anatase' and 'rutile'. Anatase TiO_2 particles are reactive and have an effect on cellular function of Arabidopsis (*Arabidopsis thaliana*) (Wang et al. 2011). In this study, we selected the more inert 'rutile' form of TiO_2 fine particles for particle exposure to minimize chemical reactions on leaf surfaces and inside the leaves. Particles were propelled towards the sapling in a wind flow generated by an axial fan. The axial fan was controlled by a frequency converter to generate the required wind speed (1, 3 or 6 m s^{-1}). Particle mass concentration (µg m^{-3}) in the tunnel was determined from filter collections done in each run. The particle size distribution (15-750 nm) was measured with a scanning mobility particle sizer (SMPS) equipped with a condensation particle counter (CPC 3025A, TSI, USA) and a differential mobility analyzer (DMA 3081, TSI, USA).

142 2.2. Plant material

143 One-year-old silver birch (*Betula pendula*) saplings were provided by the Natural Resources
144 Institute Finland, Suonenjoki research unit, and one-year-old pubescent birch (*Betula pubescens*)
145 saplings were obtained from Taimityllilä Ltd., Mäntyharju, Finland. In May 2010, saplings were
146 repotted into two liter pots filled with a 2:1 (v:v) peat:sand mixture and 1 g of N:P:K (9:3.5:5) slow
147 release fertilizer and maintained in a greenhouse at the Kuopio campus of the University of Eastern
148 Finland. Saplings were watered according to their needs, and fertilized weekly with 0.1% N:P:K
149 (19:4:20). After two weeks in the greenhouse saplings were transported to growth chambers where
150 light and temperature conditions were adjusted to represent a typical light:dark rhythm and
151 temperature of June in central Finland (Skarp et al. 1983). Lights went on at 01:00 and off at 23:00
152 reaching a maximum PAR level of 375 µmol m^{-2} s^{-1} between 06:00-18:00. The chamber
153 temperature reached its maximum of 19 ºC at 10:00 and then started to decrease towards 12 ºC after
154 18:00 holding this temperature between 00:00-04:00. Relative humidity (RH) was adjusted to a
155 standard level of 52%.

156

157 Saplings of both species were taken individually to the wind tunnel in order to determine the effects
158 of wind speed and fine particles on particle capture efficiency and BVOC emissions. Half of the 24
159 saplings of each species were exposed to fine particles carried by the wind and the other half were
160 exposed to the wind without particles. Stomatal conductance (g_s) was measured with a porometer
161 (LI-COR, model LI-1600 Steady state porometer, USA) from one leaf per sapling before the
162 exposure and the same leaf was then measured after the exposure (Table 1).

163

Table 1. Stomatal characteristics (n = 5-12) of *Betula pendula* and *Betula pubescens* in wind tunnel exposures (average ± SE, particle treatments combined).

		Betula pendula	*Betula pubescens*
Stomatal conductance (cm s^{-1})			
Before		0.45 ± 0.21	0.49 ± 0.21
After	1 m s^{-1}	0.14 ± 0.02	0.15 ± 0.03
	3 m s^{-1}	0.11 ± 0.02	n.d.
	6 m s^{-1}	0.19 ± 0.02	0.17 ± 0.02
Stomatal density (# mm^{-2})		160 ± 5.8	107 ± 6.6
Stomatal length (µm)		23.3 ± 1.2	36.4 ± 1.8

n.d., results are missing for instrument failure.

2.3. Particles on the leaf surface and particle intake

Fine particles were identified on leaf surfaces with a field emission scanning electron microscope (SEM, Zeiss Sigma HD VP, Carl Zeiss NTS, Cambridge, UK) equipped with two 60 mm^2 energy dispersive spectrometers (EDS, Thermo Noran System 7, Madison, WI, USA). Leaf pieces of 5 mm x 5 mm from the abaxial (under) and adaxial (upper) sides of three air dried leaves from each sapling were cut and attached to aluminum stubs covered with a double sided copper tape. Samples were coated with a ca. 50 nm gold layer (Automatic Sputter Coater B7341, Agar Scientific Ltd., UK). Each leaf sample was then randomly examined with a 3000 x magnification (leaf sample area 6500 μm^{-2}) in order to count the particles.

Particle capture efficiency (C_p) was calculated by eq. 1 (Räsänen et al. 2013):

$$C_p = \frac{N}{X_u A} \quad (1)$$

where N (#) is the number of particles on the leaf surface, A (m^2) is the total leaf area analysed and X_u (# m^{-2}) is the amount of particles in the air equal to the leaf area, calculated by eq. 2:

$$X_u = c \times t \times u \quad (2)$$

where c (# m^{-3}) is the particle number concentration in the air, t (s) is the duration of the exposure and u (m s^{-1}) is the average wind speed. A particle mass concentration in the air c_m (µg m^{-3}) was measured with filter measurements and then transformed to a number concentration by eq. 3:

$$c = c_m \times k \quad (3)$$

where k (# μg^{-1}) is the mass concentration to number concentration conversion coefficient determined from simultaneous filter and SMPS measurements. Deposition velocity (V_g, cm s^{-1}) was calculated with eq. 4 (Räsänen et al. 2013):

$$V_g = Cp \times u \quad (4)$$

200 The abaxial side of the leaf samples were micrographed with a 500 x magnification from three
201 random spots. Stomatal density was determined with the ImageJ program (Schneider et al. 2012) by
202 counting the number of stomata from an area of ca. 0.20 mm^2 of each spot (Table 1). Length of the
203 stomata (including guard cells) was calculated from 15 randomly selected stomata that were
204 micrographed from each sapling with a 4000 x magnification (Table 1).

205

206 Samples for studying the stomatal intake of TiO_2 particles by transmission electron microscopy
207 (TEM) were prepared from *B. pendula* leaves exposed to particles at wind speeds of 1 m s^{-1} and 3 m
208 s^{-1}. One of the youngest fully grown leaves of each tree (*n*=5 for both wind speeds) was detached
209 and a 1.5 x 1 mm leaf segment was cut next to the central vein in the middle of the leaf in a drop of
210 cold prefixative (2.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.2). The segments were kept
211 overnight in the prefixative (+ 4 °C), then rinsed with 0.1M phosphate buffer (pH 7.2), postfixed in
212 1% osmiumtetroxide (prepared in 0.1M phosphate buffer) for 3 hours, rinsed with the buffer,
213 dehydrated in an increasing ethanol series, treated with propylene oxide and embedded in Ladd's
214 epon. Several thin sections from each sample were cut with a microtome (Reichert Jung Ultracut E,
215 Austria). Sections were placed on copper grids and stained with uranyl acetate and lead citrate to
216 give contrast. TEM sections (studied using JEOL JEM2100F, Tokyo, Japan, operating at 200 kV)
217 consisted of adaxial and abaxial epidermis, palisade and spongy tissues and occasionally cross-
218 sections of veins. Electron dense globules inside the leaves were identified using EDS analysis
219 (Thermo Noran System 7, Madison, WI, USA).

220

221 2.4. Sampling of BVOCs

222

Directly after the wind tunnel runs, the saplings were moved back to a growth chamber (conditions as above) for BVOC measurements. A pre-cleaned (120 °C for 60 min) polyethylene terephthalate (PET) cooking bag was carefully closed round the uppermost side branch of the sapling, and bags were filled with air that had been passed through an ozone scrubber (Ozone Scrubber Cartridge, Environnement S.A., France) and an activated carbon filter (Wilkerson F03-C2-100, Mexico). Air was channelled through Teflon tubing and entered the bags at a small hole cut in the upper corner at a rate of 600 ml min^{-1}. The bags were filled to a constant volume and the flow was maintained until the air inside the bags had been displaced three times. A hole was then cut at the second upper corner of the bag and a 6-litre air sample was pulled through an adsorbent-filled steel tube (Perkin Elmer, ATD sample tubes, filled with 150 mg Tenax TA absorbent Supelco, mesh 60/80) with a vacuum pump (Thomas 5002 12V DC) at a rate of 200 ml min^{-1}. Blank samples from empty bags without shoots were also collected. Care was taken that the bag volumes were the same for each sapling sampled and that leaves were not in contact with the inner surface of the bag. After the collection, sampling tubes were stored in a fridge and the sampled branches were scanned with a flatbed scanner (HP Scanjet 3670) for determination of the leaf area with ImageJ (version 1.48v).

VOC samples were analyzed by gas chromatography-mass spectrometry (GC-MS, Hewlett Packard GC type 6890, MSD 5973, Beaconsfield, UK). Compounds were released from Tenax by thermal desorption (Perkin Elmer ATD400 Automatic Thermal Desorption System) at 250 °C for 10 min, cryofocused in a cold trap at -30 °C and subsequently injected onto an HP-5 capillary column (50 m x 0.2 mm i.d. x 0.33 µm film thickness, J&W Scientific, Folsom, CA, USA). The temperature program was 40 °C for 1 min, followed by an increase of 5 °C min^{-1} to 210 °C and 20 °C min^{-1} to 250 °C. Helium was used as carrier gas. Twenty-nine authentic standards were used to identify mono- and homoterpenes (C10 and C11), sesquiterpenes (C15) and green leaf volatiles (GLVs).

247 VOCs without standards were identified by comparing mass spectra to ones in the Wiley library.
248 The VOC emission rates were calculated as reported in Kivimäenpää et al. (2013) and given as
249 mass per leaf area per time (ng m^{-2} h^{-1}).

250

251 2.5. Data analysis

252 Statistical analysis was done with SPSS Statistics program (IBM Corp. Released 2010. IBM SPSS
253 Statistics for Windows, Version 19.0. Armonk, NY, USA). Normality of the data was tested with a
254 Shapiro-Wilk test and homogeneity of variances with Levene's test. Main effects of species, wind
255 speed and their interaction on C_p and V_g were tested with two-way ANOVA, and the effect of
256 different wind speeds on C_p and V_g were further studied with a Polynomial contrast test. In cases
257 where both linear and quadratic contrasts were significant, the one with the smaller p-value was
258 selected. Differences in particle deposition to leaf upper and under sides were tested with paired-
259 samples T-tests. Polynomial contrast was used to test for differences in BVOC emission at different
260 wind speeds. The effect of particle exposure on BVOC emissions of each species and wind velocity
261 were separately tested by Mann-Whitney test.

262

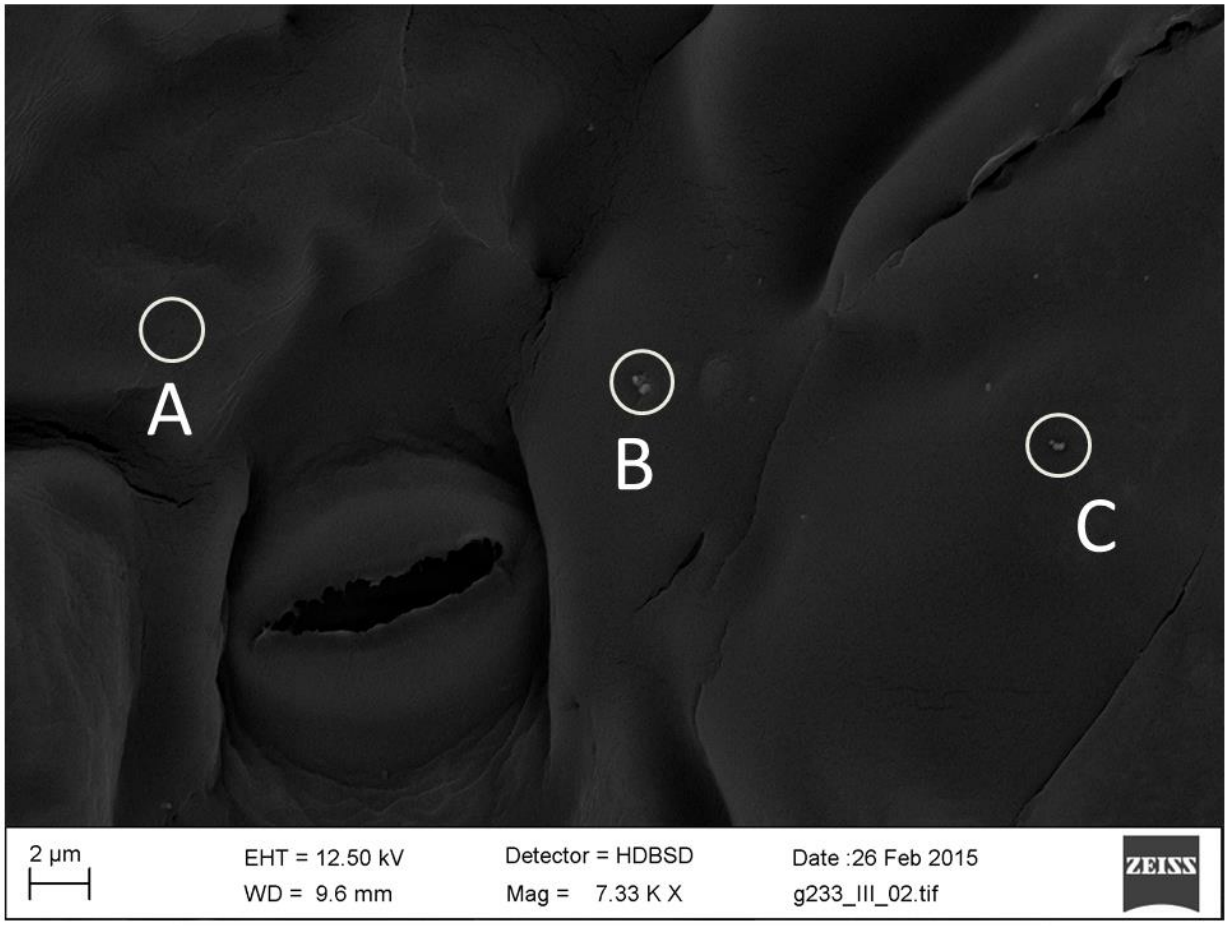
263 **3. Results**

264

265 3.1. Particle deposition on leaf surfaces

266 Particles on the leaf surfaces were observed under SEM and were typically clustered with SEM-
267 EDS analysis showing that they contained Ti and O (Fig. 1). Ti was not present in the particle-free

surface areas (Fig. 1). Other elements, including C, Au, Mg, Cu, K and Ca originated from samples, sample preparation or sample stubs and were identified in the studied sample spots.


A
B
C
2 µm
EHT = 12.50 kV
WD = 9.6 mm
Detector = HDBSD
Mag = 7.33 K X
Date :26 Feb 2015
g233_III_02.tif
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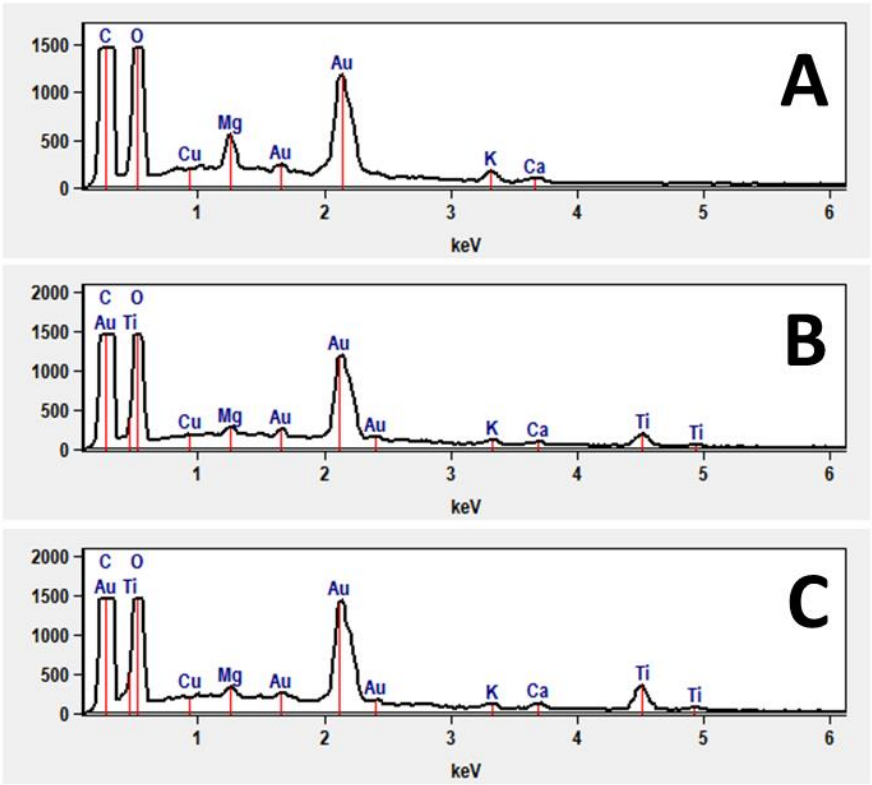
A
B
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C
O
Au
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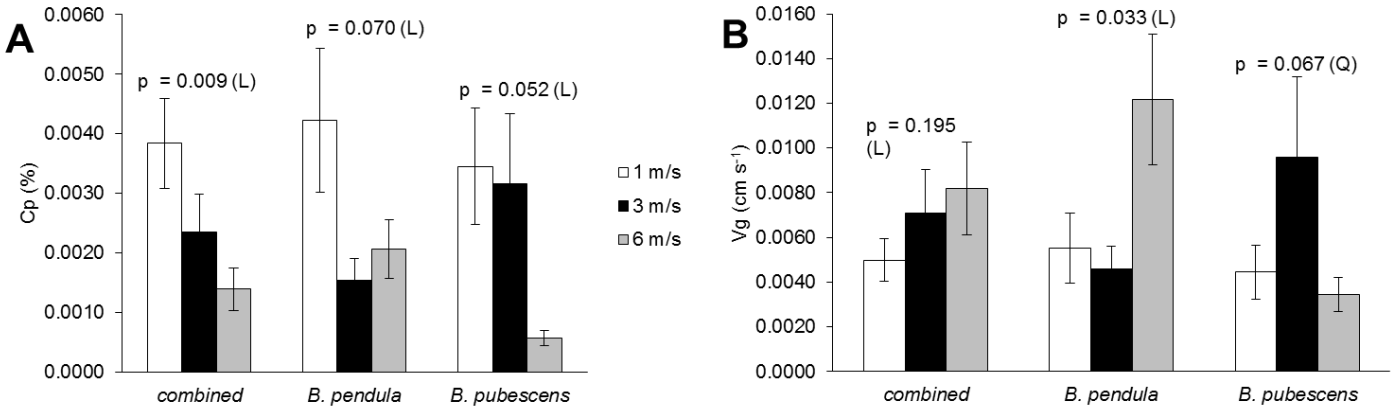
Cu
K
Ca
Ti
keV

270

Fig. 1. Background area (A) and TiO_2 particles (B, C) on leaf surfaces identified with SEM-EDS. The upper panel shows an SEM image of the *Betula pendula* leaf surface and the lower panel shows EDS spectra from the marked areas A, B and C.

Particle capture efficiency (C_p, Fig. 2A) did not significantly differ between foliage of *B. pendula* (C_p 0.0026 ± 0.0005% for all wind speeds combined) and *B. pubescens* (0.0025 ± 0.0006%) ($p > 0.05$ for the main effect of species, two-way ANOVA). Deposition velocities (V_g, Fig. 2b) were also similar for *B. pendula* (0.0074 ± 0.0014 cm s^{-1}, all wind speeds combined) and *B. pubescens* (0.0060 ± 0.0014 cm s^{-1}) ($p > 0.05$ for the main effect of species, two-way ANOVA). The species and wind speed interaction was significant for V_g ($p = 0.015$, two-way ANOVA) but not for C_p ($p > 0.050$, two-way ANOVA). C_p values decreased linearly with increasing wind speed when data for both species were combined (Fig. 2A). The decrease in C_p values with increasing wind speed was also marginally significant when both species were analysed separately (Fig. 2A). Increasing wind speed increased deposition velocity (V_g) on *B. pendula* (Fig. 2B). For *B. pubescens*, V_g values were highest at the 3 m s^{-1} wind speed (Fig. 2B). Particles were deposited more efficiently on the underside than the upper side of *B. pendula* leaves at a wind speed of 1 m s^{-1} and also when all wind speed groups were combined in the analysis (Table 2). Particle deposition on upper and under sides of *B. pubescens* leaves was similar at all studied wind speeds (Table 2).



290

291 **Fig. 2.** A) Particle capture efficiencies (C_p ± SE) and B) deposition velocities (V_g ± SE) for species
292 combined (n = 11-12), *Betula pendula* (n = 6) and *Betula pubescens* (n = 5-6) at wind speed 1 m s^{-1}
293 (white bars), 3 m s^{-1} (black bars) and 6 m s^{-1} (grey bars). Reported *p*-values represent the
294 significance of polynomial contrast tests (L = linear contrast, Q = quadratic contrast) for wind
295 speed.

296

297 **Table 2.** Particle deposition on upper and under sides of the leaves of *Betula pendula* and *Betula*

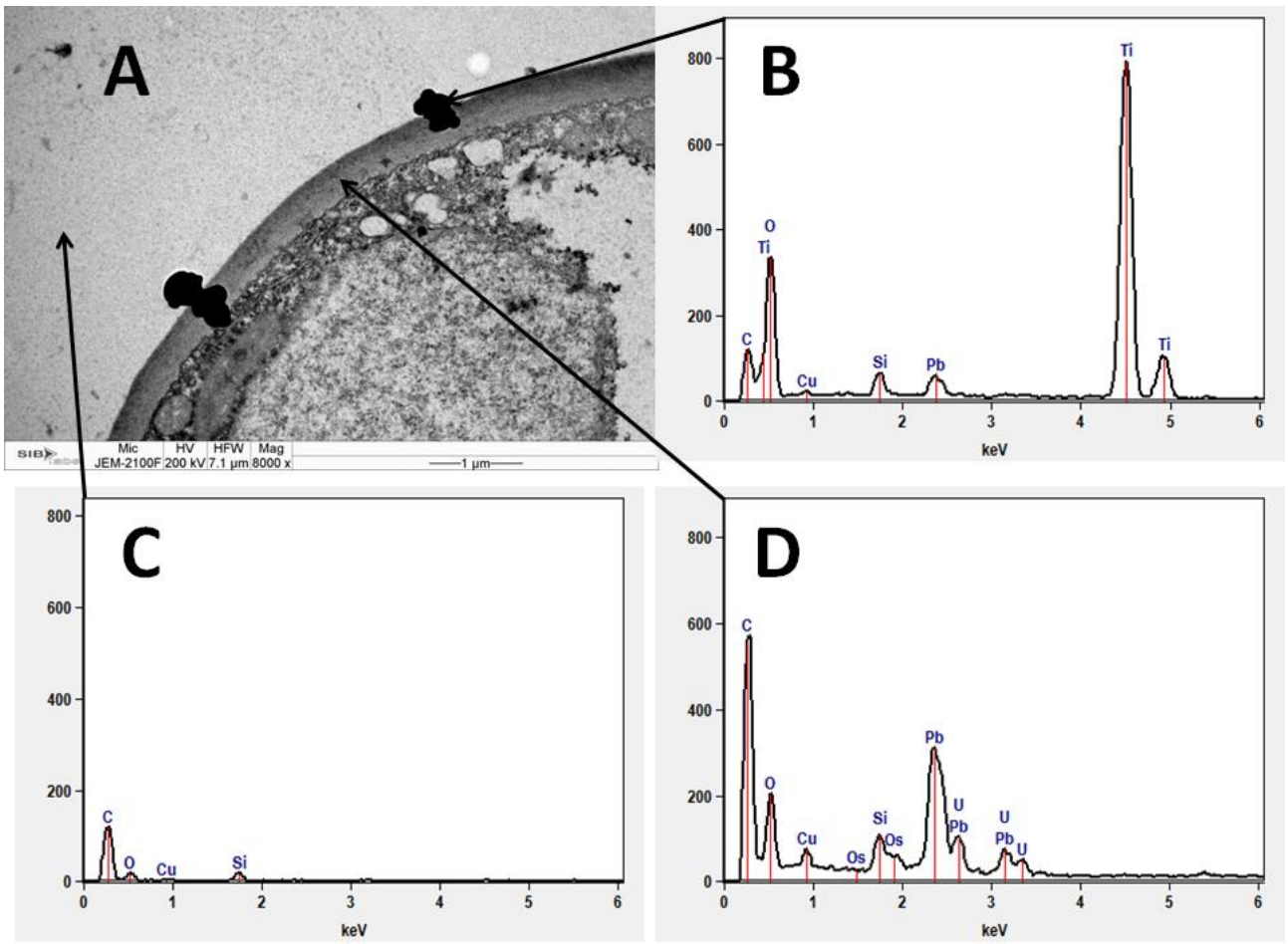
298 *pubescens*. Reported *p*-values indicate statistical significance ($p < 0.05$) of paired samples T-test.

Species	Wind speed (m s^{-1})	Leaf side		*p*	n
		Upper (%)	Under (%)		
Combined	All	43.4 ± 4.3	56.6 ± 4.3	ns	35
Betula pendula	All	34.0 ± 5.4	66.0 ± 5.4	0.009	18
Betula pubescens	All	53.2 ± 6.1	46.8 ± 6.1	ns	17
Combined	1 m s^{-1}	46.8 ± 8.8	53.2 ± 8.8	ns	12
	3 m s^{-1}	42.6 ± 7.6	57.4 ± 7.6	ns	12
	6 m s^{-1}	40.5 ± 6.4	59.5 ± 6.4	ns	11
Betula pendula	1 m s^{-1}	25.3 ± 7.2	74.7 ± 7.2	0.018	6
	3 m s^{-1}	41.9 ± 9.9	58.1 ± 9.9	ns	6
	6 m s^{-1}	34.9 ± 11.1	65.1 ± 11.1	ns	6
Betula pubescens	1 m s^{-1}	68.2 ± 10.1	31.8 ± 10.1	ns	6
	3 m s^{-1}	43.2 ± 12.4	56.8 ± 12.4	ns	6
	6 m s^{-1}	47.3 ± 4.6	52.7 ± 4.6	ns	5

299 ns = no statistical significance

3.2. Particle intake into leaves

TiO_2 particles were found in three of the five *B. pendula* leaves exposed to particles at a wind speed of 1 m s^{-1}, but in none of those exposed at a wind speed of 3 m s^{-1}. Particles identified as TiO_2 were 400-500 nm in diameter and often aggregated. They were free in the intercellular space or in contact with the cell walls of spongy mesophyll cells (Fig. 3) close to the underside of leaf surface where stomata are located. TiO_2 particles were not found elsewhere in the leaves whereas C, Au, Mg, Cu, K and Ca originating from samples, sample preparation or sample stubs were identified in the studied sample spots.



311 **Fig. 3**. Aggregated TiO_2 particles on the cell wall in the intracellular space of the underside of leaf
312 surface of *Betula pendula* at a wind speed of 1 ms^{-1} (A) and EDS charts of identified materials:
313 TiO_2 particle (B), intracellular space (C) and cell wall (D).

314

315 3.3. BVOC emission rates from *Betula pendula* and *Betula pubescens*

316

317 In total, 21 mono- and homoterpenes, 14 sesquiterpenes, 8 GLVs, and two other BVOCs were
318 found to be emitted by *B. pendula* after the wind tunnel exposure. *B. pubescens* emitted 13 mono-
319 and homoterpenes, 12 sequiterpenes, 8 GLVs and two other BVOCs. GLV compounds had the
320 highest emission rates: $1.0x10^5 \pm 4.3x10^4$ ng m^{-2} h^{-1} (33% of the total BVOC emission) for *B.*
321 *pendula* and $2.8x10^5 \pm 4.8x10^4$ ng m^{-2} h^{-1} (42% of total BVOC emission) for *B. pubescens*, cis-3-
322 hexenyl acetate being the single most emitted BVOC in both species. The most dominant
323 monoterpene emitted was sabinene by *B. pendula* ($9.3x10^3 \pm 6.0x10^3$ ng m^{-2} h^{-1}, 3% of the total
324 BVOC emission) and linalool by *B. pubescens* ($7.3x10^2 \pm 1.4x10^2$ ng m^{-2} h^{-1}, 0.1% of total BVOC
325 emission). Of the sesquiterpenes, (*E,E*)-α-farnesene had the highest emission rates in *B. pendula*
326 ($1.0x10^4 \pm 3.5x10^3$ ng m^{-2} h^{-1}, 3% of the total BVOC emission) and aromadendrene in *B. pubescens*
327 ($1.6x10^3 \pm 2.0x10^2$ ng m^{-2} h^{-1}, 0.2% of the total BVOC emission).

328

329 3.4. Effects of wind speed and particle exposure on BVOC emission rates

330

331 Table 3 shows the significant effects of wind speed on the mean emission rates of individual
332 BVOCs as well as the total emission rates of compound groups. In *B. pendula*, the general pattern

for most monoterpenes, sesquiterpenes and nonanal was that emission rates increased from 1 m s^{-1} to 3 m s^{-1} and then decreased again so that emission rates at 6 m s^{-1} were at a similar or lower level than at 1 m s^{-1}. Emission rates of the ocimenes and caryophyllene oxide decreased and the GLV cis-3-hexen-1-ol increased linearly with wind speed.

For *B. pubescens,* emission rates of nonanal and the monoterpenes β-pinene, cis-β-ocimene and linalool decreased with increasing wind speed, and unlike in *B. pendula* there was no peak in the emission rate at 3 m s^{-1}. Emission rates of the GLV 1-hexanol increased with increasing wind speed. Responses of sesquiterpenes differed between the compounds, but the overall response was that emission rates were lowest at the 3 m s^{-1} velocity, but increased again at the 6 m s^{-1} velocity to the same level as at the 1 m s^{-1} velocity. This response was also observed for the homoterpene 4,8-dimethyl-1,3,7- nonatriene (*E*-DMNT). The effects of particle exposure on BVOC emissions were not significant (data not shown).

Table 3. Emission rates [ng m^{-2} h^{-1}] of birch, total mono-, homo- and sesquiterpenes, GLVs and individual BVOCs affected by wind speed at the level $p < 0.100$ (n = 12). Values are reported as combined averages (±SE) of particle exposed and control seedlings. *p*-values of polynomial contrasts (Q = quadratic, L = linear) of univariate ANOVA are shown.

	Wind speed (m s^{-1})				
	1	3	6	*p*	Contrast
Betula pendula					
Total mono- and homoterpenes	**14 000 (3 400)**	**45 000 (24 000)**	**5 600 (1 100)**	**ns**	
α-pinene	3 000 (2 400)	5 200 (3 100)	300 (81)	0.053	Q
sabinene	700 (290)	23 000 (15 000)	300 (100)	0.035*	Q
β-pinene	860 (430)	2 400 (1 400)	220 (63)	0.058	Q
myrcene	900 (370)	1 300 (580)	350 (79)	0.064	Q
cis-β-ocimene	2 800 (670)	2 100 (700)	660 (210)	0.027*	L
limonene	830 (210)	1 600 (730)	350 (76)	0.015*	Q
α-ocimene	440 (200)	410 (160)	150 (56)	0.018*	L
Total Sesquiterpenes	**12 000 (2 100)**	**46 000 (28 000)**	**9 900 (2 300)**	**ns**	
α-copaene	300 (50)	5 600 (3 800)	1 100 (370)	0.071	Q
longifolene	460 (150)	6 500 (4 700)	700 (260)	0.049*	Q
α-humulene	210 (99)	2 100 (1 400)	310 (120)	0.041*	Q
cadinene	650 (440)	5 100 (3 600)	600 (210)	0.031*	Q
caryoph. oxide	350 (170)	110 (65)	87 (35)	0.012*	L
Total GLVs	**18 000 (12 000)**	**350 000 (210 000)**	**260 000 (110 000)**	**ns**	
cis-3-hexen-1-ol	13 000 (9 400)	37 000 (8 900)	47 000 (16 000)	0.040*	L
Total other BVOCs	**12 000 (3 200)**	**12 000 (1 700)**	**4 100 (1 100)**	**0.007***	L
nonanal	9 700 (2 100)	11 000 (1 400)	3 600 (900)	0.009*	L
Betula pubescens					
Total mono- and homoterpenes	**2 500 (500)**	**1 300 (260)**	**1 100 (220)**	**0.098**	L

β-pinene	130 (48)	73 (26)	43 (16)	0.045*	L
cis-β-ocimene	100 (25)	35 (11)	79 (43)	0.001*	Q
linalool	1 400 (320)	590 (180)	270 (100)	0.009*	L
E-DMNT	250 (83)	90 (28)	290 (100)	0.002*	Q
Total Sesquiterpenes	**7 400 (1 500)**	**4 100 (980)**	**6 700 (1 100)**	**0.006***	Q
α-copaene	170 (54)	68 (18)	93 (19)	0.002*	Q
longifolene	930 (430)	190 (60)	2.5 (70)	0.024*	Q
trans-β-farnesene	1 100 (200)	1 100 (390)	1 600 (340)	0.022*	Q
aromadendrene	1 300 (240)	1 200 (250)	2 200 (430)	0.052	Q
α-humulene	480 (170)	260 (69)	420 (92)	0.027*	Q
α-farnesene	2 100 (560)	920 (310)	1 300 (340)	0.016*	Q
germacrene	67 (54)	67 (54)	79 (35)	0.063	Q
Total GLVs	**590 000 (200**	**440 000 (99 000)**	**930 000 (310 000)**	**ns**	
1-hexanol	47 (33)	1 000 (330)	8 000 (4 600)	0.060	L
Total other BVOCs	**5 000 (1 100)**	**2 000 (270)**	**2 000 (170)**	**0.002***	**L**
nonanal	4 500 (1 100)	1 800 (220)	1 900 (160)	0.001*	L

*$p < 0.050$, statistically significant

ns not significant at level $p < 0.1$

4. Discussion

4.1. Foliar deposition and intake of particles

Particle deposition to leaf surfaces was one order of magnitude lower here than in our previous studies conducted with *B. pendula* and *B. pubescens* (Räsänen et al. 2013). However, similar C_p values were reported in an earlier study of *Quercus robur* L. and *Eucalyptus globulus* with a larger particle size (Freer-Smith et al. 2004; Reinap et al. 2009). The particle size studied here was less than half the size used in the previous study of *B. pendula* and *B. pubescens* (0.27 µm vs. 0.7 µm) (Räsänen et al. 2013), which probably explains most of the differences observed in the C_ps because the minimum particle size for deposition is around 0.3 µm (Hinds 1999; Grantz et al. 2003). In contrast, species differences (e.g. a smooth surface structure not favoring particle uptake) and differences in experimental set-up might be the reason for the low C_p values of *Quercus robur* L. and *Eucalyptus globulus* (Freer-Smith et al. 2004; Reinap et al. 2009). Furthermore, differences in the methods to determine particle capture might have some effect on the C_p values determined. Previously, we analyzed mass of salt particles deposited on leaf surfaces whereas in the current study we determined the number of deposited particles by SEM analysis. The previous method emphasized the deposition of larger particles (mass on leave surface) and the current method the deposition of smaller particles (number). Räsänen et al. (2013) reported that *B. pubescens* captured more particles than *B. pendula*, which could be due to species-related qualities, such as hairiness of leaves. The particle size used in this study is complex because it is too large for efficient Brownian diffusion, but too small for impaction (Hinds 1999; Grantz et al. 2003), which can explain the equal C_p values of both birch species. This means that the leaf surface structure characteristics of the birch species studied might be less important in determining capture of this particle size. Furthermore,

383 particles were found on both sides of the leaves, which supports determining the C_p by using the
384 total leaf area (see Räsänen et al. 2013).

385

386 The C_p values of this study were lower at higher wind speeds, which is in line with the known
387 behavior of ultrafine particles (Lin and Khlystov 2012). Typically, a particle diameter of 0.5 µm has
388 been considered a threshold after which larger particles have more inertia and deposition increases
389 with increasing wind speed (Beckett et al. 2000). There is also a possibility for salt particles to
390 become deliquescent, thus increasing C_p values (Burkhardt et al. 2001), but this is not likely to
391 happen with inert, water insoluble TiO_2 particles. The decreasing C_p with increasing wind speed
392 was clearest when all replicates were combined, and only marginally significant when species were
393 analysed separately which suggests that the number of replicates should be increased in future
394 experiments to reduce variation. The effects of wind speed on V_g were not so clear, but values were
395 more likely to increase with faster wind speed.

396

397 Although particle deposition to leaf surfaces was generally low, the finding that inert TiO_2 particles
398 sometimes penetrate inside the leaf structure is very interesting. The real opening of stomata of *B.*
399 *pendula* was not measured, but the stoma length of over 20 µm would enable these fine particles to
400 penetrate through the stomata into intercellular space. Previous studies have shown water-
401 suspended particles of 43 nm and 1.1 µm in diameter to penetrate through the stomata of *Vicia faba*
402 (Eichert et al. 2008), which are similar in size to those of the *B. pendula* used in this study. To our
403 knowledge, this is the first study to report stomatal uptake of solid airborne fine particles. Direct
404 uptake of particles through stomata should be studied further for a better understanding of the
405 nanomaterial pathway in the food chain.

406

407 4.2. BVOC emissions

408

409 The wind speed that the birches were exposed to before BVOC sampling affected the BVOC
410 emission rates and the chemical profiles, with various compounds or compound groups having
411 different responses. Moreover, responses in the two birch species differed in their relation to wind
412 velocity. For example, our results indicated the highest emission rates of several BVOCs to be at a 3
413 m s^{-1} wind speed in *B. pendula* and to decrease at a higher wind speed, while emission rates of
414 several BVOCs decreased linearly for *B. pubescens*. The reasons for the differences between the
415 species may be in the leaf structure. For *B. pendula*, a wind speed of 3 m s^{-1} might have disturbed
416 glandular trichomes, storage structures of volatile terpenoids (Biswas et al. 2009), which were
417 reported to have six times greater density in *B. pendula* than *B. pubescens* (Räsänen et al. 2013),
418 whereas a wind speed of 6 m s^{-1} might have been strong enough to ensure that a large quantity of
419 trichome stored BVOCs were released before the measurement was done. The linear increase in a
420 few dominant GLV compounds with increasing wind speed in both species may indicate increased
421 membrane-damage due to mechanical stress (Vuorinen et al. 2004; Mithöfer et al. 2005). Wind
422 could also affect emission rates of water-soluble BVOCs (e.g. GLV) by affecting stomatal
423 conductance (Niinemets et al. 2004; Harley et al. 2014). However, the role of stomatal function
424 could not be confirmed in this study since stomatal conductance dropped to the same level in all
425 wind speed treatments after the test runs, and change in stomatal conductance during the wind
426 tunnel experiment was not possible to monitor (cf. Räsänen et al. 2012). This was the first study to
427 show that the prevailing wind speed before sample collection could affect BVOC emissions of birch
428 trees. However, further studies are needed before recommendations of tree selection for improving
429 air quality at e.g. windy or calm locations can be made. One reason is the large variation in BVOC

composition and emission rates of plant individuals and chemotypes (Hakola et al. 2001, Bäck et al. 2012, Kivimäenpää et al. 2013), which was also seen in this study.

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