Eesti Maaülikool
Estonian University of Life Sciences

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HAAVA (*Populus sp.*) ISOPREENI EMISSIOONILE

ZHIHONG SUN

A Thesis
for applying for the degree of Doctor of philosophy
in Plant Physiology

Väitekiri
filosoofiadoktori kraadi taotlemiseks taimefüsioloogia erialal

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Opponent: **Dr. Carlo Calfapietra**
Institute of Agro-Environmental and Forest Biology (IBAF), National Research Council (CNR), Monterotondo (Rome) - Porano (TR)

Supervisor: **Prof. Ülo Niinemets**
Institute of Agricultural and Environmental Sciences Estonian University of Life Sciences Tartu, Estonia

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CONTENTS

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The Present of thesis is based on the following papers, which are referred to in the text by Roman numerals:

I. **Sun Z,** Niinemets Ü, Hüve K, Rasulov B, Noe SM. (2013) Elevated atmospheric CO_2 concentration leads to increased whole-plant isoprene emission in hybrid aspen (*Populus tremula* x *P. tremuloides*). *New Phytologist,* 198, 788-800.

II. **Sun Z,** Niinemets Ü, Hüve K, Noe SM, Rasulov B, Copolovici L, Vislap V. (2012) Enhanced isoprene emission capacity and altered light responsiveness in aspen grown under elevated atmospheric CO_2 concentration. *Global Change Biology,* 18, 3423-3440.

III. **Sun Z,** Hüve K, Vislap V, Niinemets Ü. (2013). Elevated growth [CO_2] enhances isoprene emissions under high temperatures and improves thermal resistance in hybrid aspen. (Submitted to *Global Change Biology*).

IV. **Sun Z,** Copolovici L, Niinemets Ü. (2012) Can the capacity for isoprene emission acclimate to environmental modifications during autumn senescence in temperate deciduous tree species *Populus tremula*? *Journal of Plant Research,* 125, 263-274.

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The contributions of the authors to the papers:

Paper	Idea and study design	Data collection	Data analysis	Manuscript preparation
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II	**ZH,** ÜN	**ZH,** KH ,VV, BR	**ZH,** ÜN,BR	**ZH,** ÜN
III	**ZH,** ÜN	**ZH,** VV,	**ZH,** ÜN	**ZH,** ÜN
IV	ÜN, LC, **ZH**	**ZH,** LC	**ZH,** ÜN, LC	**ZH,** ÜN, LC

BR - Bahtijor Rasulov; KH - Katja Hüve; LC - Lucian Copolovici; SN - Steffen M. Noe; ÜN - Ülo Niinemets; VV - Vivian Vislap; ZH - Zhihong Sun.

DEFINITIONS OF ABBREVIATIONS AND SYMBOLS

ABBREVIATION	DEFINITION
ATP	Adenosine triphosphate
BVOCs	Biogenic volatile organic compounds
DMADP	Dimethylallyl diphosphate
DXP	1-deoxy-D-xylulose-phosphate
DXP pathway	1-deoxy-D-xylulose-phosphate pathway
DXR	1-deoxy-d-xylulose 5-phosphate reductoisomerase
GA3P	Glyceraldehyde-3-phosphate
IDP	Isopentenyl diphosphate
ISPS	Isoprene synthase
MDA	Malonyldialdehyde
MVA	Mevalonic acid
MVA pathway	Mevalonic acid pathway
NO	Nitric oxide
NOx	Oxides of nitrogen
NPQ	Non-photochemical quenching
$^{\bullet}OH$	Hydroxyl radicals
PAN	peroxyacetyl nitrate
PEP	Phosphoenolpyruvate
Pyr	Pyruvate
ROS	Reactive oxygen species
RuBP	Ribulose-1,5-bisphosphate
SOA	Secondary organic aerosol
VOCs	Volatile organic compounds
Ambient (380)	Grown under ambient measured at [CO_2] 380 µmol mol^{-1}
Ambient (780)	Grown under ambient measured at [CO_2] 780 µmol mol^{-1}
Elevated (380)	Grown under elevated measured at [CO_2] 380 µmol mol^{-1}
Elevated (780)	Grown under elevated measured at [CO_2] 780 µmol mol^{-1}

SYMBOL	DEFINITION	UNITS
$A_{max,}$	Light-saturated net assimilation rate	µmol m^{-2} s^{-1}
C_A	Leaf carbon content per leaf area	g m^{-2}
C_i	Intercellular CO_2 concentration	µmol mol^{-1}
$C_{i,max}$	Intercellular CO_2 concentration for maximum isoprene emission rate	µmol mol^{-1}
C_M	Leaf carbon content per dry mass	%
$I_{max,c}$	Isoprene emission rate at $C_{i,max}$	nmol m^{-2} s^{-1}

$I_{max,Q.}$	Light-saturated isoprene emission rate	nmol m^{-2} s^{-1}
J_{max}	Maximum photosynthetic electron transport rate	µmol m^{-2} s^{-1}
M_A	Leaf dry mass per unit area	g m^{-2}
M_C	Whole canopy leaf dry mass	g $plant^{-1}$
N_A	Leaf nitrogen content per leaf area	g m^{-2}
N_M	Leaf nitrogen content per dry mass	%
$R_{C:N}$	Carbon to nitrogen ratio of foliage	
$V_{c,max}$	Maximum carboxylation rate of Rubisco	µmol m^{-2} s^{-1}

1. INTRODUCTION

Isoprene (2-methyl-1, 3-butadiene) is the most abundant and dominant biogenic volatile organic compound emitted from a wide range of plant species (Monson *et al.*, 2012, Sharkey *et al.*, 2008). Isoprene accounts for 40% of total global biogenic volatile organic compounds (BVOCs) emissions with the total amount emitted of 440–660 Tg carbon year^{-1} (Guenther *et al.*, 2006). As a dominant and highly reactive BVOC, isoprene reacts rapidly with hydroxyl radicals (•OH), nitrogen oxides (NO_X) and ozone in atmosphere after release from vegetation; this strongly impacts tropospheric ozone production, aerosol formation and partly controls the methane lifetime (Kanakidou *et al.*, 2005, Pacifico *et al.*, 2009). Thereby, isoprene emissions potentially influence large-scale Earth system processes (Fehsenfeld *et al.*, 1992; Claeys *et al.*, 2004; Hallquist *et al.*, 2009), thus it is essential to investigate the influence of environmental factors on isoprene emissions from plants to predict global climate change and provide useful guidelines on environmental management policies for future.

It has been previously demonstrated that isoprene plays an important role in protecting plants from biotic and abiotic stresses. In particular, isoprene emission can enhance thermotolerance by direct stabilization of membranes and perhaps by reducing heat-induced damage of photosynthetic apparatus under light as evidenced by maintenance of lower non-photochemical quenching (NPQ) at given light level (Behnke *et al.*, 2010, Sharkey *et al.*, 2001a). In fact, isoprene seems to be involved in maintenance of the macro-organization of the pigment-protein complexes in membranes during thermal stress (Velikova *et al.*, 2011). Isoprene also can quench reactive oxygen species (ROS) caused by stress (Affek *et al.*, 2002, Brilli *et al.*, 2007, Brüggemann *et al.*, 2002, Loreto *et al.*, 2001b), and it has been speculated that isoprene exerts its protective action at the membrane level by quenching stress-produced ROS, and thereby maintaining biomembrane stability (Affek *et al.*, 2002, Vickers *et al.*, 2009). However, despite the great progress in resolving the role of isoprene in plants, the isoprene protection mechanism is still unclear.

Isoprene is formed in chloroplasts by isoprene synthase (ISPS) from its direct precursor dimethylallyl diphosphate (DMADP). Chloroplastic DMADP is primarily synthesized through 1-deoxy-D-xylulose-

phosphate (DXP) pathway (Affek *et al.*, 2003, Lichtenthaler, 1999, Schwender *et al.*, 2001). Isoprene formation is tightly bound to leaf photosynthetic carbon metabolism, which provides glyceraldehyde-3-phosphate (GA3P) and ultimately also pyruvate for DMADP formation (Affek *et al.*, 2003, Delwiche *et al.*, 1993, Trowbridge *et al.*, 2012), although the origin of the latter metabolite in chloroplasts is less clear. Labelling experiments have found that multiple carbon sources contribute to isoprene formation when photosynthesis is limited by environmental stress (Brilli *et al.*, 2007, Funk *et al.*, 2004, Trowbridge *et al.*, 2012). However, as yet, the physiological mechanisms of regulation of isoprene synthesis are still not be fully resolved.

Isoprene emission is highly variable and is affected over short term by changes in light and temperature, and over long term by preceding weather factors and by plant growth stage and ontogeny (Kuhn *et al.*, 2004; Loreto *et al.*, 1993, Trowbridge *et al.*, 2012). Development of isoprene emission capacity is related to maturation of leaf photosynthetic competence as young leaves are incapable of isoprene emission due to lack of ISPS activity (Kuhn *et al.*, 2004, Kuzma *et al.*, 1993). In addition, the ISPS gene displays diurnal and seasonal variation in expression (Mayrhofer *et al.*, 2005), but the regulation of isoprene synthesis in leaves undergoing senescence is poorly understood. In addition, under moderate stress, isoprene emissions are usually enhanced, even if photosynthesis is significantly suppressed (Vickers *et al.*, 2009, Niinemets *et al.*, 2010d, Loreto *et al.*, 2010). Yet, severe stress can significantly impair isoprene emission possibly reflecting limiting carbon availability for isoprene synthesis (Vickers *et al.*, 2009, Niinemets *et al.*, 2010d, Loreto *et al.*, 2010). The extent to which stressed and old leaves can maintain isoprene emission despite reduced carbon availability has not yet been fully resolved.

The rise of atmospheric [CO_2] is one of the most important global-scale issues. It has been predicted that rising [CO_2] results in global warming with more severe drought and higher temperatures in summer (Norby *et al.*, 2004, Walther *et al.*, 2002). On the other hand, current atmospheric [CO_2] is far below the [CO_2]-saturation point of photosynthesis. Thus, an increase in atmospheric [CO_2] per se is expected to increase the rates of photosynthesis and plant growth (Ainsworth *et al.*, 2005, Long *et al.*, 2004, Luo *et al.*, 2006). Greater carbon availability under higher [CO_2] in turn may increase plant stress tolerance (Niinemets, 2010b).

Atmospheric [CO_2] rise is also potentially an important factor influencing global isoprene emissions, especially given the possible interactions of [CO_2] increase with other key environmental drivers (Tuba *et al.*, 2007, Calfapietra *et al.*, 2010). Effects of growth [CO_2] on isoprene emissions have been investigated in different plant species, but the results have been controversial, making it difficult to extrapolate to future conditions. Most past studies conducted with different species have found that elevated [CO_2] results in a significant inhibition of isoprene emissions (Darbah *et al.*, 2010, Monson *et al.*, 1989, Monson *et al.*, 2007, Possell *et al.*, 2011, Rosenstiel *et al.*, 2003, Scholefield *et al.*, 2004, Sharkey *et al.*, 1991). In contrast, several other studies have shown no or moderate impact of growth [CO_2] (Loreto *et al.*, 2007, Calfapietra *et al.*, 2008, Sharkey *et al.*, 1991). These controversial outcomes may reflect differences in plant growth conditions, differences in the experiment duration and interspecific variability in elevated [CO_2] effects (Sharkey *et al.*, 1991, Taub *et al.*, 2008, Tognetti *et al.*, 1998). Furthermore, few studies have investigated the combined effects of elevated [CO_2] on leaf-level isoprene emission and canopy development (e.g., Possell and Hewitt, 2009). Yet, this is critical because elevated [CO_2] may increase canopy leaf area, thereby possibly offsetting the leaf-level inhibition. Also, possible modifications in leaf-age dependent changes in isoprene emission by elevated [CO_2] or by [CO_2] induced global warming and changes in stress resistance may alter whole canopy integrated isoprene emission rate.

The main purpose of this thesis was to study the effects of elevated atmosphere CO_2 concentration and plant ontogenetic stage on isoprene emissions from aspen [European aspen (*Populus tremula*) and hybrid aspen (*P. tremula* x *P. tremuloides*)] in order to improve the understanding of how acclimation to elevated [CO_2] on alters isoprene emissions through plant development at leaf and canopy scales. We hypothesized that: (1) Elevated [CO_2] stimulates leaf area growth and leads to increased canopy isoprene emission rates; (2) Elevated [CO_2] alters leaf-level isoprene emission rates by both modifying instantaneous [CO_2] response and due to long-term acclimation to [CO_2]; (3) Acclimation to elevated [CO_2] changes isoprene emission responses to other environmental drivers such as light; (4) Elevated [CO_2] acclimated plants have greater heat stress resistance, despite possibly lower leaf-level isoprene emission rates at their growth [CO_2] environment; (5) Leaf capacity to adjust to changes

in temperature environment decreases with increasing leaf age. The hypotheses (1-4) were examined in 2-year-old hybrid aspen (*P. tremula* x *P. tremuloides*) plants. Isoprene emission was studied at canopy and leaf scales from the start of canopy development to maturation under different ambient [CO_2]; Leaf chemical and structural traits, photosynthetic and isoprene emission characters, DMADP pool size and ISPS activity and heat stress responses were studied to assess the acclimation effects to growth [CO_2]. The hypothesis (5) was investigated in adult European aspen (*P. tremula*) trees from leaf maturation to senescence by studying modifications in leaf chemistry, isoprene emission and photosynthetic rates, and DMADP pool size.

2. REVIEW OF LITERATURE

2.1. Vegetation source of isoprene

Volatile organic compounds (VOCs), usually defined as carbon-based non-methane chemicals with high volatility at normal temperature and pressure, are emitted into the atmosphere by anthropogenic and natural sources (Guenther *et al.*, 1995). Globally, it is estimated that around 1300 Tg VOCs is released into the atmosphere yearly; the majority are biogenic volatile organic compounds (BVOCs), predominantly coming from terrestrial ecosystems such as forests, grass-, shrub- and croplands (Goldstein *et al.*, 2007, Guenther *et al.*, 1995, Guenther *et al.*, 2006, Kiendler-Scharr *et al.*, 2009).

Isoprene (2-methyl-1, 3-butadiene) was first discovered as a cell metabolite from plants by Sanadze in 1950s (Sanadze, 2004) and was independently discovered by Rasmussen in 1970s by gas chromatography with mass spectroscopy (Sharkey *et al.*, 2001b). Isoprene is among the most abundant and predominant BVOCs emitted from vegetation by wide range of plant species, e.g. angiosperms, gymnosperms, ferns and mosses (Monson *et al.*, 2012, Sharkey *et al.*, 2001b). Its share may reach up to 40% of total global BVOCs emission with rates of 440–660 Tg C $year^{-1}$ (Guenther *et al.*, 2006). Many broadleaf trees are high isoprene emitters, such as poplars, oaks, and eucalypts; it is estimated that worldwide around half of isoprene is emitted from tropical broadleaf trees and the remainder is released from temperate trees and shrubs (Guenther *et al.*, 2006).

2.2. Isoprene in atmospheric photochemistry

Isoprene plays a major role in troposphere oxidation chemistry because of its largest source and high reactivity for reactions with hydroxyl radicals ($^{\bullet}OH$), ozone, and NO_x (Guenther *et al.*, 2006, Paulot *et al.*, 2009). Isoprene reaction with $^{\bullet}OH$ coincides with peaks in $^{\bullet}OH$ production during the day time; and these reactions of VOCs with $^{\bullet}OH$ constitute the dominant pathway for removal of many pollutants and greenhouse gases (Kwan *et al.*, 2012, Taraborrelli *et al.*, 2009). By reacting with $^{\bullet}OH$, isoprene is converted into peroxy radicals. These peroxy radicals can

be further oxidized by •OH or other oxidative radicals under clean air conditions, or oxidized by nitric oxide (NO) under polluted conditions (Paulot *et al.*, 2009). Thus, by removing •OH from the atmosphere, reactive VOCs lead to the increased lifetime of CH_4 (Lelieveld *et al.*, 2008).

Isoprene can be directly oxidised by O_3 and NO_3. Under little polluted or clean conditions (low NOx) isoprene is oxidized by O_3, thus the O_3 concentration decreases. Under NOx polluted conditions, isoprene oxidation at high NO concentrations produces NO_2 which increases O_3 level as the result of photolysis of NO_2 leading to formation of mono-oxygen that rapidly reacts with O_2 to form O_3 (Pacifico *et al.*, 2009, Taraborrelli *et al.*, 2009). Thus, isoprene affects the rates of tropospheric ozone production and destruction.

The oxidation of isoprene by atmospheric radicals forms enormous amounts of peroxides (~100 Tg C yr^{-1}) and isoprene nitrates, like PAN (peroxyacetyl nitrate, $RC(O)OONO_2$), organic nitrates ($RONO_2$); these can be incorporated into aerosols (SOA, <3%) or can be deposited, being thus, significant in atmospheric carbon and nitrogen budgets (Henze *et al.*, 2006, Kroll *et al.*, 2006, Paulot *et al.*, 2009). In return, the formation of atmospheric particles will impact radiation balance of earth by scattering or absorbing light and participating in cloud formation as cloud condensation nuclei (Kulmala *et al.*, 2004). Thus, isoprene emissions have far-reaching impact on air quality, global tropospheric chemistry and climate change, implying that it is essential to investigate the impacts of environmental factors on isoprene emissions to predict global climate change and propose environmental management policies for future.

2.3. Isoprene biosynthesis in plants

In high plants, DMADP and its isomer, isopentenyl diphosphate (IDP), are basic five-carbon atom precursors for all isoprenoids. Two independent metabolic pathways are responsible for the biosynthesis of these larger isoprenoids, plastidic DXP pathway and cytosolic mevalonic acid (MVA) pathway (Bick *et al.*, 2003, Lichtenthaler *et al.*, 1997). In plastidic pathway, DMADP/IDP biosynthesis which starts from pyruvate and glyceraldehyde-3-phosphate (GA3P) is responsible for the formation

of isoprene, monoterpenes, diterpenes, carotenoids and plastoquinone. MVA-dependent isoprenoid biosynthesis pathway starts from 3 acetyl-CoA and is located in the cytosol. This pathway is mainly responsible for formation of sesquiterpenes, triterpenes, sterols and ubiquinones (Bick *et al.*, 2003, Lichtenthaler, 1999). There is a certain crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in leaves at the level of IDP that can be transported between plastids and cytosol (Bick *et al.*, 2003, Schnitzler *et al.*, 2004).

It is well established that isoprene as a natural hemiterpene is predominantly biosynthesized in chloroplasts from its immediate precursor dimethylallyl diphosphate (DMADP) catalyzed by isoprene synthase (ISPS) (Lichtenthaler *et al.*, 1997, Schwender *et al.*, 2001). Thus, DMADP availability and isoprene synthase activity are key factors determining the isoprene emission rate (Calfapietra *et al.*, 2008, Rasulov *et al.*, 2009b, Rasulov *et al.*, 2010, Schnitzler *et al.*, 2004). Isoprene emission depends on light and temperature and is affected by environmental and leaf external factors. $^{13}CO_2$ labelling experiments have revealed that in unstressed plants, most of the carbon in emitted isoprene was derived from recently assimilated photosynthates (Karl *et al.*, 2002, Trowbridge *et al.*, 2012).

However, the contribution of newly fixed carbon flow to isoprene production significantly decreased under heat or water stress, and isoprene emission was sustained when photosynthesis was almost completely inhibited under most severe phase of stress (Brilli *et al.*, 2007, Funk *et al.*, 2004). Stored carbon can be mobilized to maintain isoprene formation under stressed conditions, such as chloroplastic starch breakdown, xylem-transported carbohydrates, and cytoplasmic pyruvate/phosphoenolpyruvate equivalent (Funk *et al.*, 2004, Laule *et al.*, 2003, Trowbridge *et al.*, 2012). Labelling experiments have confirmed contribution of multiple carbon sources to isoprene formation when photosynthesis is limited by stress. This evidence implies that there is a dynamic exchange of carbon between different intermediates of isoprene biosynthesis, and an increasing importance of these alternative carbon pools to maintain isoprene formation under conditions of limited photosynthesis (Bick *et al.*, 2003, Schnitzler *et al.*, 2004).

Recent studies have found that the isoprene synthase gene encoding ISPS displays diurnal and seasonal variations in expression (Loivamäki *et*

al., 2007, Mayrhofer *et al.*, 2005). In young or developing leaves, both MVA and DXP pathway are mainly involved in formation of higher molecular mass terpenoids and pigments, including carotenoids, phytol, plastoquinone and ubiquinone, which are primarily incorporated in chloroplast and mitochondrial membranes (Lichtenthaler, 2007, Wiberley *et al.*, 2009). In grey poplar leaves, ISPS activity can not be detected until the foliage is close to physiological maturity (Mayrhofer *et al,* 2005). In mature leaves, ISPS activity is strongly dependent on leaf age and is also correlated with day-to-day variations in temperature and light (Loivamäki *et al.*, 2007, Wiberley *et al.*, 2009). The study from Cinege *et al.* (2009) reported that the environmental responsiveness of isoprene formation over days to weeks is linked to light and temperature, which directly modulates the isoprene synthase promoter activity. In a tropical tree *Hymenaea*, isoprene emission rate declined in the leaves of recently matured to senescent leaves (Kuhn *et al.*, 2004), indicating the determination of isoprene emission by ontogenetic and environmental factors. Great progress has been made in understanding isoprene biosynthesis and regulation of ISPS, however, physiological regulation mechanisms of isoprene synthesis, shared between ISPS activity and DMADP pool size, are still not fully resolved.

2.4. Biological roles of isoprene emission

Isoprene emission is a substantial cost to the plant in terms of both carbon and energy. However, previous experiments have confirmed that plants obtain substantial physiological benefits by isoprene emission in particularly when subjected to abiotic stresses, like high temperature and reactive oxidant stress (Niinemets *et al.*, 2010c, Sharkey *et al.*, 2008). The role of isoprene in protecting against thermal stress has been relatively well studied. The first evidence for thermotolerance was found based on a photosystem II chlorophyll fluorescence assay (Sharkey *et al.*, 1996). The subsequent studies via either fumigating non-isoprene emitting plants with isoprene or by feeding DXP pathway inhibitor fosmidomycin to the isoprene emitting species, revealed that both endogenous or exogenous isoprene can improve plants thermal tolerance and relieve the suppression of photosynthesis in heat-stressed leaves (Loreto *et al.*, 2001b, Singsaas *et al.*, 1997). Transgenic *Arabidopsis* plants overexpressing *Populus alba* ISPS, were observed to have significantly improved heat tolerance and decreased leaf surface temperature (Sasaki

et al., 2007). Behnke *et al.* (2007) further reported reduced efficiency of photochemistry and increased non-photochemical quenching (NPQ) in grey poplar leaves where ISPS gene was silenced, indicating greater need for NPQ-dependent dissipation of excess energy.

The function of isoprene emission in antioxidative defence has also been observed. The ozone fumigation experiments in reed (*Phragmites australis*) plants showed that isoprene prevents ozone-driven destruction of the photosynthetic apparatus by quenching the amount of H_2O_2 and reducing malonyldialdehyde (MDA) concentration and lipid peroxidation in cellular membranes (Loreto *et al.*, 2001a, Velikova *et al.*, 2004). It has been reported that isoprene fumigation of non-emitting young leaves decreased the photosynthesis depression caused by singlet oxygen produced by the photosensitizer Rose Bengal (Affek *et al.*, 2002). The protective functions of isoprene were observed also in other stress experiments. For instance, isoprene emission reduced the limitation of drought and salinity on photosynthesis (Brilli *et al.*, 2007, Brüggemann *et al.*, 2002). These results indicated that isoprene may reduce oxidant-induced oxidative damage and inhibit accumulation of toxic ROS caused by stress (Vickers *et al.*, 2009).

Furthermore, it has been reported that isoprene emitted from transgenic *Arabidopsis* expressing isoprene synthase from grey poplar interferes with the attraction of bodyguards by herbaceous plants (Loivamäki *et al.*, 2008). Investigations with transgenic isoprene-emitting tobacco found that isoprene emissions influenced herbivore feeding decisions in Manduca sexta caterpillars (Laothawornkitkul *et al.*, 2008). These two lines of evidence suggest that isoprene might be a repellent for specialized insects. It has further been reported that isoprene significantly accelerated the onset of flowering when crop plants were exposed to exogenic isoprene with 50-150 ppbv (Terry *et al.*, 1995), suggesting that isoprene may be involved in developmental interactions.

All abiotic stresses result in production of reactive oxygen species (ROS) (Vickers *et al.*, 2009), and once their level is over a certain threshold, the excess of ROS will be largely accumulating, resulting in injury or damage, like changes in specific lipid–protein interactions and dynamic properties of the lipid bilayers (Vickers *et al.*, 2009, Yoshioka *et al.*, 2008). This leads to impaired electron transport via PSI and PSII and

photophosphorylation (Hald *et al.*, 2008), and membrane leakage and damage of photosynthetic systems (Mahajan *et al.*, 2005, Wang *et al.*, 2008b). Recently finding from Velikova *et al.* (2011) showed that isoprene maintained macro-organization of the pigment-protein complexes in the membranes, and also stabilized light-induced transmembrane electric field and recombination of the PSII donor and acceptor side charges. Despite the major progress, the protection mechanism of isoprene is still unclear. The evidence suggests that isoprene protects plants by multiple mechanisms, mainly at the level of membranes owing to its high lipid solubility and high reactivity with ROS.

2.5. Isoprene emissions responses to environmental drivers light, temperature, and soil resources

Isoprene emission is affected by growth environmental conditions and plants' ontogenetic stage. It has been observed that isoprene emission capacity starts to develop just before full leaf photosynthetic competence; after reaching a maximum emission rate, isoprene emission capacity starts to decline accompanied with foliage senescence (Kuzma *et al.*, 1993, Monson *et al.*, 1994, Kuhn *et al.*, 2004). Such seasonal variations of isoprene emission are combined with changes in DMADP pool size, 1-deoxy-d-xylulose 5-phosphate reductoisomerase (DXR) and ISPS gene expression and corresponding proteins amounts and enzymes activities (Loivamäki *et al.*, 2007, Mayrhofer *et al.*, 2005, Willmer *et al.*, 2009). Thus, the regulation of isoprene biosynthesis occurs on multiple levels and is associated with modulation of environmental factors and foliage ontogeny (Cinege *et al.*, 2009, Monson *et al.*, 1994, Sasaki *et al.*, 2005).

Under non-stressed or moderate stress conditions, isoprene emission is tightly connected to photosynthesis. The emission falls to negligible rates almost immediately after the cessation of illumination, associated with the stopped supply of energy and carbon substrate from recently fixed CO_2 (Loreto *et al.*, 1993, Trowbridge *et al.*, 2012). The hyperbolic dependence of isoprene emissions on light is qualitatively similar to the light dependency of photosynthesis, and especially to that of photosynthetic electron transport (Harley *et al.*, 1996, Monson *et al.*, 1989, Niinemets *et al.*, 1999). Under strong light intensity above photosynthetic saturation point, isoprene emission is still stimulated (Monson *et al.*, 1989, Sharkey *et al.*, 1993), suggesting that isoprene

emission becomes partly uncoupled from the photosynthesis under extreme light conditions.

Temperature is another important factor strongly affecting the rate of isoprene emission (Singsaas *et al.*, 1999). Isoprene emission rate exponentially increase with temperature until to an optimum temperature at approximately 45 °C, and subsequently rapidly decreases with further temperature increase (Harley *et al.*, 1996, Li *et al.*, 2011, Rasulov *et al.*, 2010). The responses of isoprene emissions to temperature are currently not entirely understood. The study from Rasulov *et al.* (2010) reported that the temperature-dependent decrease at around 45 °C was caused by decreases in DMADP concentration and ISPS activity, and proposed that the reduction of DMADP pool size was caused by decreased energy supply (ATP) generated in photosynthesis. In natural field conditions, the situation is further complicated by interaction of high temperature spells with drought.

However, isoprene emission rate is less sensitive than photosynthesis to water stress. Studies indicate that under moderate drought, isoprene emission is unaffected or only slight decreases, though photosynthesis obviously is strongly decreased (Brilli *et al.*, 2007, Fang *et al.*, 1996, Loreto *et al.*, 1993). It was observed that when soil water limitation caused photosynthesis to decline even to zero after several days of drought, isoprene emissions still maintained at appreciably high values (Brilli *et al.*, 2007, Brüggemann *et al.*, 2002, Centritto *et al.*, 2011). Analogously, it has been reported that salt stress affects isoprene emission slightly though it reduces the photosynthesis severely (Loreto *et al.*, 2000, Teuber *et al.*, 2008). Yet, newly fixed carbon incorporation into isoprene was lower when photosynthesis was depressed by drought (Brilli *et al.*, 2007). These results indicate that multiple alternative carbon sources can contribute to isoprene emission when photosynthesis is constrained by stress (Brilli *et al.*, 2007, Funk *et al.*, 2004, Trowbridge *et al.*, 2012). However, under severe stress, isoprene emission is strongly reduced and this is associated with reduction of ISPS protein concentration and activity (Fortunati *et al.*, 2008).

Soil nutrition supply can also affect isoprene emission (Litvak *et al.*, 1996, Loreto *et al.*, 2000, Teuber *et al.*, 2008). Previous studies found positive correlations between isoprene emission rate and leaf nitrogen

concentration (Litvak *et al.*, 1996, Teuber *et al.*, 2008), suggesting that increased soil nitrogen supply may lead to higher isoprene emission capacity.

2.6. Isoprene emission responses to elevated [CO_2]

Since the preindustrial revolution, atmospheric [CO_2] has been increased from 280 μmol mol^{-1} to a current approximately 390 μmol mol^{-1} and predicted to continue to rise to as much as 500 –1000 μmol mol^{-1} by the year 2100 (de Graaff *et al.*, 2006, Long *et al.*, 2004, Luo *et al.*, 2006). It was predicted that rising of [CO_2] will result an increase of the mean temperature of the Earth by a few degrees (Long *et al.*, 2004, Norby *et al.*, 2004). Thus, warming will affect plants' growth stage and it may enhance global evaporation and bring about drought and high temperature stresses in summer (Walther *et al.*, 2002).

With the continuous rising of atmospheric [CO_2], the effects of elevated [CO_2] on isoprene emission becomes particularly important. The effects of CO_2 concentration on isoprene emission rate have been reported since 1960s by Sanadze, showing that isoprene emission rate from the leaves of poplar trees decreased as atmospheric [CO_2] increased (Sharkey *et al.*, 2001b, Wilkinson *et al.*, 2009). In past decades, effects of elevated [CO_2] on isoprene emission have been studied from different plant species and under different experimental conditions. However, there exist controversial results among the studies. Most investigations reported that elevated growth [CO_2] decreased isoprene emission rate, e.g. *Populus tremuloides* (Darbah *et al.*, 2010, Sharkey *et al.*, 1991, *P. deltoides* (Rosenstiel *et al.*, 2003), *Phragmites australis* (Scholefield *et al.*, 2004), *Liquidambar styraciflua* (Monson *et al.*, 2007, Wilkinson *et al.*, 2009), *Acacia nigrescens* (Possell *et al.*, 2011), and *Platanus orientalis* (Velikova *et al.*, 2009). Other studies demonstrated that elevated [CO_2] did not significantly affect the isoprene emission rate (Calfapietra *et al.*, 2008, Centritto *et al.*, 2004, Loreto *et al.*, 2007). Furthermore, in some investigations, long-term elevated [CO_2] treatment increased foliage isoprene emission capacity (Sharkey *et al.*, 1991, Tognetti *et al.*, 1998).

The instantaneous response of isoprene to CO_2 concentration is characterized by an asymmetric curve with an optimum at low intercellular CO_2 concentration (C_i) close to the CO_2 compensation

point of photosynthesis, below which isoprene emission decreases rapidly and above which isoprene emissions decreases relatively slowly (Rasulov *et al.*, 2009a). A double carboxylation scheme has been proposed such that ribulose-1,5-bisphosphate (RuBP) carboxylase and phosphoenolpyruvate (PEP) carboxylase control CO_2 carboxylation in chloroplast (GA3P production) and cytosol (pyruvate production), thereby affecting the substrate supply to the DXP pathway (Sanadze, 2010, Monson *et al.*, 2009). On the other hand, it was believed that inorganic phosphate become bound to sugar phosphates when CO_2 concentration is rising, thereby curbing ATP synthesis (feedback inhibition) and leading to reduction of isoprene formation due to limited energy status (Loreto *et al.*, 1993, Rasulov *et al.*, 2009b). Yet, the exact mechanism of isoprene emission response to instantaneous CO_2 is still not known well, although different hypotheses are in agreement that the reduction of isoprene emission under higher CO_2 concentration is due to reduced DMADP pool size.

Currently, the atmospheric [CO_2] is far below the CO_2 saturation point of photosynthesis. Thus the effects of elevated [CO_2] on plants are multifaceted, including enhancement carbon uptake and net primary production, which alter canopy structure and leaf anatomical traits, and modulate the adaptation processes associated with environmental stress (Ainsworth *et al.*, 2003, Calfapietra *et al.*, 2010, Taub *et al.*, 2000, Wang *et al.*, 2010). Almost in all the investigations, elevated [CO_2] has increased the photosynthesis rate when measured at elevated [CO_2] (Ainsworth *et al.*, 2005, Leakey *et al.*, 2009). On the other hand, many studies have found that long-term exposure to elevated CO_2 led to a reduction of photosynthetic capacity via the decrease of maximum carboxylation rate of Rubisco ($V_{c,max}$) and maximum electron transport rate (J_{max}), reflecting reduced RuBP regeneration rate (Ainsworth *et al.*, 2007, Chen *et al.*, 2005, Zhang *et al.*, 2009). However, other experimental evidence indicated that there was no down-regulation of photosynthetic capacity and the plants grown long-term (several months to years) under elevated [CO_2] treatment had higher or unaffected J_{max} and V_{cmax} (Ainsworth *et al.*, 2003, Calfapietra *et al.*, 2005, Liberloo *et al.*, 2007). In particular, legume species show greater enhancement of photosynthesis and growth, and less down regulation by elevated CO_2 compared those un- legumes plants (Rogers *et al.*, 2009), suggesting that soil N nutrition may importantly affect the responses to growth [CO_2] as

enhanced N supply may abolish the down-regulation in photosynthetic capacity caused by elevated [CO_2].

On the other hand, previous investigations have observed that plants grown under elevated [CO_2] had greater heat stress tolerance of photosynthesis and had higher water use efficiency, measured either under greenhouse or field conditions (Ainsworth *et al.*, 2005, Centritto *et al.*, 1999, Lin *et al.*, 2002, Sanz-Saez *et al.*, 2010). Leaves formed at elevated [CO_2], maintained higher PSII efficiency (F_v/F_m) and lower photorespiration to higher temperatures than ambient-[CO_2]-grown leaves (Huang *et al.*, 2007, Taub *et al.*, 2000). Thus, long-term elevated [CO_2] treatment alters plants' physiological characteristics and sensitivity to other environmental factors. The key question explored in this Thesis was whether plants will alter their isoprene emission characteristics associated with altered physiological potentials after long-term elevated [CO_2] acclimation?

2.7. Modelling isoprene emission responses to rising atmospheric [CO_2]

As isoprene emissions are likely affected by future rise of atmospheric [CO_2], and also be influenced by possible more frequent drought and thermal stresses during the growing season, it is important to quantitatively simulate isoprene emission responses to global change drivers. Many models that predict isoprene emission from plants are based on empirical or semi-mechanistic algorithms (Guenther *et al.*, 1993; Niinemets *et al.*, 1999; Guenther *et al.*, 2006; Heald *et al.*, 2009). These models usually utilize leaf-scale measurements and rely on meteorological input parameters as driving factors (Arneth *et al.*, 2008a, Pacifico *et al.*, 2009). Thus, elevated CO_2 will stimulate greater vegetation productivity, including enhanced leaf area and mass; furthermore, rising atmospheric [CO_2] combined with warming can result in longer growth period (Long *et al.*, 2004, Luo *et al.*, 2006). Therefore, it has been predicted that elevated atmospheric [CO_2] will lead to enhanced isoprene emissions globally in future (Arneth *et al.*, 2008b, Guenther *et al.*, 1995, Wiedinmyer *et al.*, 2006). However, the models using empirical parameterization based on measurements of instantaneous responses to elevated [CO_2] (Wilkinson *et al.*, 2009) suggested that the emissions may decrease in future higher [CO_2] atmospheres at global scale (Arneth

et al., 2007, Heald *et al.*, 2009, Monson *et al.*, 2007, Young *et al.*, 2009). These large differences in estimates of potential future changes in isoprene emission lie in uncertainties and complexities of elevated [CO_2] multifunctional effects, including instantaneous and long-term acclimation and even combinations with other environmental factors (Pacifico *et al.*, 2009). These uncertainties lead to different predictions in modelling future climate (Young *et al.*, 2009). Therefore, it is essential to study the effects of elevated atmosphere [CO_2] and plant ontogenetic stage on isoprene emissions, in order to improving the understanding of acclimation effects of elevated [CO_2] and natural temperature variations on isoprene emission through plant development.

3. AIMS OF THE STUDY

The overall objective of this thesis is to study the influence of environmental factors on isoprene emission from strong isoprene emitter aspen [European aspen (*Populus tremula*) and hybrid aspen (*P. tremula* x *P. tremuloides*)] in order to gain insight into possible modifications in isoprene emission due to climate change. In particular, focusing on the effects of elevated atmospheric [CO_2] and natural variation in temperature. Though the stimulation of leaf area growth may largely compensate for possible [CO_2]-inhibition reported at leaf scale, combined effects of elevated [CO_2] on dynamic canopy development, net assimilation and isoprene emission rates have rarely been studied.

Given the large and poorly understood study-to-study variations in elevated [CO_2] responses of isoprene emission, we studied the dynamics of canopy leaf area development and net assimilation and isoprene emission rates at canopy and leaf scales in hybrid aspen from bud-burst to full canopy development. The plants were grown at [CO_2] of 380 μmol mol^{-1} (ambient) and 780 μmol mol^{-1} (elevated) to test the hypothesis that elevated [CO_2] stimulates leaf area growth, thereby potentially leading to increased canopy isoprene emission rates. To further examine whether leaf-level isoprene emissions are reduced in plants grown under elevated [CO_2], and if so, why, foliar isoprene emission and photosynthesis characteristics, DMADP pool size and ISPS activity were studied in hybrid aspen after long-term growth under elevated [CO_2].

Long-term elevated [CO_2] effects on plant physiology are multifaceted, but currently there are few studies on acclimation of isoprene emission capacity associated with plant abiotic stress tolerance. Responses of isoprene emission and net assimilation rates and membrane leakage to heat stress were investigated in hybrid aspen grown under ambient and elevated [CO_2], to test the hypotheses that elevated [CO_2] acclimated plants have a greater resistance to extreme temperatures, and that this response is associated with enhancement of isoprene emissions under heat stress despite possible downregulation under lower temperatures.

Isoprene emission capacity is modulated by leaf age and strongly affected by variations in long-term temperature. In future climates, growing season for deciduous species is potentially longer, implying also greater

leaf longevity for plants with deterministic leaf growth as, for example, is the case in mature trees of aspen. There are few studies on isoprene emission changes during leaf aging and the way leaves of different age acclimate to temporal changes in environmental drivers such as day-to-day and week-to-week differences in average temperature. Especially limited is the information for the period of leaf senescence. We examined the changes in temperature adaptation kinetics in dependence on leaf age in mature trees of European aspen, and tested the hypothesis that the capacity for acclimation of isoprene synthesis potentials decreases with increasing leaf age.

The specific aims of this thesis were:

(1) To test the hypothesis that elevated [CO_2] stimulation of leaf area growth leads to increased canopy isoprene emission rates. To examine the effects of elevated growth [CO_2] on plant ontogenetic process, canopy leaf area development, and canopy photosynthetic and isoprene emission dynamics in hybrid aspen (**Paper I**);

(2) To separate the short- and long-term controls of elevated [CO_2] on isoprene emission, and test the hypothesis that growth under elevated [CO_2] alters both the short-term (instantaneous) and long-term (acclimation) CO_2 responses of isoprene emission (**Papers II-III**);

(3) To separate the controls on isoprene emission by its substrate DMADP and isoprene synthase activity in plants grown under different [CO_2] concentrations, and test the hypothesis that possible modifications in DMADP pool size alter isoprene emission responses to other environmental drivers such as light, thereby altering the light use efficiency for isoprene biosynthesis (**Paper II**);

(4) To gain insight into the possible mechanisms of heat protection by isoprene, and regulation of isoprene emission during thermal stress. In particular, testing the hypothesis that plants acclimated to elevated [CO_2] have a greater heat stress resistance due to greater heat-induced isoprene emission (**Paper III**);

(5) To examine the effects of long-term variations in environmental temperature and foliage aging on the capacity of isoprene emission throughout leaf aging (**Paper IV**).

4. MATERIALS AND METHODS

4.1. Plant material (Papers I-IV)

Saplings of hybrid aspen (*Populus tremuloides* Michx. x *P. tremula* L.) were selected to examine the effects of elevated [CO_2] on isoprene emissions (Paper I, II and III). Hybrid aspen is a cross between European aspen (*Populus tremula* L.*)* and North-American trembling aspen (*Populus tremuloides* Michx.) (Haikio *et al.*, 2009). Many clones have been selected based on faster growth compared to native aspen (*Yu et al.*, 2003). The clones H55 and H200 are widely used in Estonian and Finnish forestry as fast growing forestry species, and their growth has also been shown to be little sensitive to ozone (Haikio *et al.*, 2009, Oksanen *et al.*, 2001). In this study, two-year old ca. 20 cm tall plants of clones H55 and H200 were used in different investigations. Before the start of the experiment, saplings were kept in a cold room at -2 ºC in dormant state, then potted in 3 L plastic pots filled with sand and peat mixture (1:1) and installed in the open gas-exchange/plant growth system for different [CO_2] treatments.

For the Paper IV, native European aspen (*Populus tremula*) trees growing in the vicinity of Tartu were used. The trees were naturally-regenerated and 8-10 years old and 8-12 m tall at the time of the experiment. Isoprene emission measurements from summer to autumn till foliage senescence were conducted in individual leaves.

4.2. The open gas-exchange system for plant growth and isoprene and photosynthesis measurements (Papers I-III)

The open whole-plant gas-exchange system was used to grow the plants under different [CO_2] conditions (Papers I, II and III), and study whole plant photosynthesis and isoprene emission through plant development under different environmental conditions (Paper I). The open gas-exchange system consisted of four individual glass chambers of 12.5 L volume (diameter 20 cm, height 40 cm) and was specially designed for plant growth and long-term continuous monitoring of plants gas-exchange activities. The principle of the flow system has been previously described by Copolovici and Niinemets (2010) and is for the full system described in detail in Papers I-III.

Chambers 1 and 3 were kept at a CO_2 concentration (average ± SD) of 380 ± 10 μmol mol^{-1} (denoted as ambient), while chambers 2 and 4 were treated with an elevated CO_2 concentration of 780 ± 10 μmol mol^{-1} (denoted as elevated). The air temperature of chambers was maintained at 28-30/23 °C for day/night, and air humidity at 60 %. Each individual chamber was lit by four 50 W halogen lamps providing a light intensity of 800 μmol m^{-2} s^{-1} on the top of the chambers for a photoperiod length of 12 hours (for detailed description see Papers I-III).

The gas stream for each chamber was divided between a reference flow corresponding to the air before entering the chamber, and a sample flow, corresponding to the gas leaving the plant chamber. LI-7000 CO_2/H_2O analyser (Li-Cor Inc., Lincoln, NE, USA.) and a fast isoprene sensor (FIS, Hills-Scientific, Boulder, CO, USA) were combined to measure online photosynthesis characteristics and isoprene emission during plants' canopy development in the chambers (for detailed description see Papers I-II)

4.3. Online monitoring of dynamics of plants growth and gas-exchange from canopy to leaf level (Paper I)

Based on the online measurements by LI-7000 CO_2/H_2O analyzer and FIS, instantaneous whole plant net assimilation and isoprene emission rates were determined for individual chambers. After integration of the instantaneous rates, daily integrated canopy net assimilation (CO_2, $A_{C,day}$) and isoprene emission ($I_{C,day}$) rates were obtained. In addition, net assimilation (A) and isoprene emission rates (I) per unit leaf area (downscaled rates, see section 4.4 for consideration of self-shading) were calculated by dividing the whole plant rates by the leaf area on the given day.

4.4. Modelling the dynamics of canopy leaf area, net assimilation and isoprene emission rates (Paper I)

The Chapman-Richards function (Bertalanffy, 1957; Liu *et al.*, 2003; Pienaar *et al.*, 1973) is a widely used growth model. This model is based on the assumption that both, the plant physiological state and the environment affect its growth pattern. In this analysis, it was assumed

that canopy leaf area development, and net assimilation and isoprene emission rates follow the Chapman-Richards function:

$$y(t) = y_0 + \lambda(1 - e^{-rt})^c, \qquad \text{(Eq. 1)}$$

where $y(t)$ is the state of the measured variable at time t, y_0 is the size of the growing component or rate of the process at time $t = 0$, λ is the maximum increase of the growing resource, r is the relative growth rate, and c determines the curve shape. Furthermore, based on Eq. 1, growth or physiological process parameters at the point of inflexion (t_i, y_i) and corresponding fastest growth or physiological process rate of R_i were calculated.

At the leaf level, we assumed that the Chapman-Richards function is still valid. Considering the self-shading during the canopy development, we introduced the Lambert-Beer law into Chapman-Richards function, expressed as:

$$z(t) = z_0 + \left(-\ln\left(\frac{Q(t)}{Q_0} \right) \cdot \lambda\left(1 - e^{-rt}\right)^c \right). \qquad \text{(Eq. 2)}$$

The parameter Q_0 reflects the quantum flux density at half-height of the chamber when the plant was installed, $Q(t)$ is the transmitted light at time t, and z_0 denotes the offset of the changing trait. Q_0 was fixed at 400 µmol m^{-2} s^{-1}, and $Q(t)$ was altered linearly in time as the canopy foliage area was increasing. The values of given physiological processes at the point of inflexion (t_i, y_i) and corresponding fastest changes of the process rates, R_i, were calculated as for the canopy processes.

4.5. Non-destructive canopy leaf area estimation during canopy development (Paper I)

Leaf area growth during the experiment was assessed by combining a non-destructive method using digital photography and destructive harvesting at the end of the experiment. The plants in the chambers were photographed daily at a fixed time from exactly the same position. Using GIMP (The GNU Image Manipulation Program, Version 2.6, www.gimp.org) program, and the silhouette leaf area was calculated from each photograph. Additional 28 plants with different canopy

size were selected to track the canopy leaf area development during the experiment. Based on these data, we got a linear relationship between the scanned real leaf area and the silhouette of the canopy leaf area estimated from each photograph. Using this regression line, true plant canopy leaf area was estimated from silhouette leaf area for any date from the start of the experiment.

4.6. Study of acclimation of leaf-level photosynthetic and isoprene emission characteristics to growth [CO_2] (Paper II)

After 30-40 days growth in the open growth/gas-exchange system, the plants were temporarily moved out to measure the photosynthetic and isoprene emission response curves to CO_2 and light and assess the effects of acclimation to growth [CO_2] on these key environmental response curves. A Walz GFS-3000 gas exchange fluorescence system equipped with a LED-Array/PAM-Fluorometer 3055-FL (Walz GmbH, Effeltrich, Germany) and FIS were used for these measurements. The measurements were conducted at leaf temperature of 30 ^{o}C and relative humidity of 60%, corresponding to growth conditions. Data labelled as Ambient (380) and Elevated (380) correspond to measurements in plants grown under ambient and elevated [CO_2], but both measured at the same leaf chamber [CO_2] of 380 µmol mol^{-1}, while, Ambient (780) and Elevated (780) correspond to plants grown under ambient and elevated [CO_2], but both measured at same leaf chamber [CO_2] of 780 µmol mol^{-1}.

Light response curves of photosynthesis were fitted by a non-rectangular hyperbola (Ögren *et al.* 1993), and light-saturated net assimilation rate (A_{max}), curvature (K), and the initial quantum yield (Φ_J) were obtained. Isoprene emission vs. light response curves were fitted by the light response function of modified Guenther *et al.* isoprene emission model (Guenther *et al.*, 1993, Niinemets, 2010a), and the light-saturated isoprene emission rate ($I_{max,Q}$), isoprene emission rate at Q = 1000 µmol m^{-2} s^{-1} (I_{Q1000}) and the quantum yield (α_T) of isoprene emission were obtained.

The CO_2 response curves of photosynthesis and isoprene emission were measured under growth light intensity of 500 µmol m^{-2} s^{-1}. Farquhar *et al.* (1980) photosynthesis model was used to fit net assimilation rate (A) vs. intercellular CO_2 (C_i) response curves by a non-linear least squares

fitting procedure. Modified Weibull type response function (Yang *et al.*, 1978 with modifications outlined in Paper II) was used to fit I vs. C_i responses. The maximum carboxylase activity of Rubisco (V_{cmax}), the capacity for photosynthetic electron transport (J_{max}), maximum isoprene emission rate ($I_{max,Ci}$) and corresponding intercellular CO_2 concentration ($C_{i,max}$) were estimated.

In addition, we employed the method of rapid light-dark transients of Rasulov *et al.* (2009a, 2010) to determine in vivo DMADP pool size and isoprene synthase rate constant. The method is based on integration of isoprene emission rate following rapid darkening of the leaves (Rasulov *et al.* 2009a, Rasulov *et al.*, 2010).

4.7. Isoprene emission responses to heat stress resistance in plants grown under different [CO_2] (Paper III)

Elevated [CO_2] affects foliage photosynthesis and isoprene emission characteristics and therefore might alter plant response to stress and potential competition in future climates. Walz GFS-3000 combined with FIS was used to compare the heat stress sensitivity of photosynthesis and isoprene emission rates for plants grown under different [CO_2]. Leaf net assimilation rate, isoprene emission rate, and carbon lost due to isoprene emission were measured. Leaf temperature was changed in the order of 30 °C → 35 °C → 40 °C → 45 °C → 50 °C, with an hold time of 8 min at each individual temperature. The measurements were conducted both at chamber CO_2 concentrations of 380 µmol m^{-2} s^{-1} and 780 µmol m^{-2} s^{-1}.

4.8. Changes in isoprene emission and photosynthesis rates during autumn senescence (Paper IV)

The twigs of mature European aspen (*Populus tremula*) trees (see section 4.1) were cut under water and transported immediately to the laboratory for photosynthesis, isoprene emission, DMADP pool size, and methanol emission measurements. The measurements were carried out in every three days between Aug. (fully active dark green leaves) to Oct. (yellowed leaves close to abscission, intensive leaf fall in the canopy) to investigate seasonal temperature change on foliage photosynthesis and isoprene and methanol emission during senescence (Niinemets *et al.*,

2010e). An infra-red dual-channel gas analyzer (CIRAS II, PP Systems, UK) was used for photosynthesis measurements, while isoprene and methanol emissions were measured with a Proton Transfer Reaction-Mass Spectrometer (PTR-MS) (Ionicon GmbH, Innsbruck, Austria) using the temperature-controlled gas-exchange system as described in Copolovici and Niinemets (2010).

Total leaf pool size of the immediate isoprene precursor, dimethylallyl diphosphate (DMADP), was determined using the acid hydrolysis technique of Fisher *et al.* (2001) with the modifications of Rasulov *et al.* (2009a).

4.9. Leaf anatomical, morphological and chemical analyses (Papers I-IV)

In this thesis, for all the investigations, after foliage physiological measurements were completed, leaf area, fresh and dry mass, and foliage nitrogen and carbon contents were measured. A Vario MAX CNS analyser (Elementar Analysensysteme GmbH, Hanau, Germany) was used to determine foliage C and N contents.

In addition, fully mature leaves were sampled for anatomical analyses. The samples were dehydrated in ethanol series and embedded in Epoxy embedding medium. Semi-thin cross-sections for light microscopy and ultra-thin cross- and paradermal sections for transmission electron microscopy (TEM) were used to take digital images (Tosens *et al.*, 2012). Leaf thickness, mesophyll thickness, chloroplast numbers and starch grain size were determined.

5. RESULTS

5.1. Effects of elevated [CO_2] on the dynamics of canopy development and gas-exchange from canopy to leaf level (Paper I)

During the process of canopy leaf development, plants grown under elevated [CO_2] had higher leaf area compared with those grown under ambient [CO_2] (Fig. 1). Elevated [CO_2] resulted in higher maximum canopy leaf area (L_A) of 942 cm^2, which exceeded that in ambient [CO_2]-grown plants (660 cm^2) by 43% (Table 1, Fig. 1). For plants grown under elevated [CO_2], the relative growth rate was 0.18 day^{-1}, which was increased by 38% than that of 0.13 day^{-1} in plants grown under ambient [CO_2] (Table. 1). Furthermore, in plants grown under elevated [CO_2], the fastest growth rate occurred at 12th day with 67 cm^2 day^{-1}. This is two days earlier and twice higher than that in plants grown under ambient [CO_2] (Table 1).

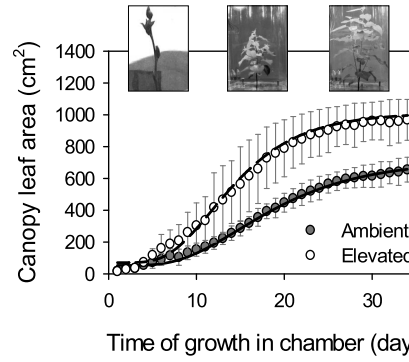


Figure 1. Growth of the plant canopy leaf area under ambient (grey circles) and elevated (white circles) [CO_2] over 5 weeks. Data are shown as circles with error bars denoting the standard deviations (mean ± SD). The solid line denote ambient and the dashed line elevated [CO_2] modelled canopy leaf area as fitted by non-linear least square regressions (Eq. 1) to the daily integrated data (r^2>0.98, P<0.0001). (Reproduced from Paper I).

Table 1. Canopy leaf area development parameters (means ± SE) according to the Chapman-Richards model (Eq. 1) (Modified from **Paper I**).

	y_0	L_A	r	c	t_i	L_i	R_i
	cm^2	cm^2	day^{-1}		day	cm^2	cm^2 day^{-1}
Ambient	52±8	660±54	0.13±0.011	6.5±1.3	14±1	220±19	33.6±3.2
Elevated	71±6	942±59*	0.18±0.021*	8.2±0.8*	12±1	323±20*	67±9*

The parameters are defined as: the offset (y_0), the maximum canopy leaf area (L_A), the relative growth rate (r) and the empirical parameter (c). The time (t_i) and leaf area (L_i) and corresponding maximum growth rate (R_i) at the inflexion point of the fitted curve are also shown. n = 10 for all parameters, and * denotes a significant difference between the mean values at P < 0.05 (paired t-tests).

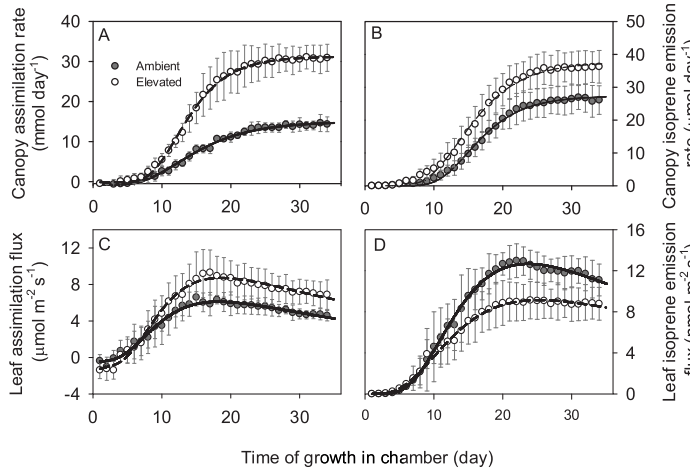


Figure 2. Dynamics of measured and modelled canopy scale daily net assimilation rate and daily isoprene emission rate (A and B), and leaf-scale net assimilation flux and isoprene emission flux (C and D). The lines denote non-linear least square regressions Eq. 1 for A and B, Eq. 2 for C and D (all $r^2 > 0.98$, $P<0.0001$). The solid lines denote ambient and the dashed lines for elevated [CO_2] treatments. Symbols and line styles are according to Fig. 3 (Modified from **Paper I**).

On the canopy scale, both integrated daily net assimilation and isoprene emission rates followed the dynamics of leaf area growth, and the Chapman-Richards function (Eq. 1) provided excellent fit to the time-courses of both processes (Fig. 2A, B, $r^2>0.98$, $P<0.0001$ in both cases). Canopy net assimilation rate increased faster and reached at an almost double maximum value ($A_{max,canopy}$) of 32 mmol day^{-1} under elevated than under ambient [CO_2] (16 mmol day^{-1}; Fig. 2A, Table 2). Elevated [CO_2] resulted in approximately three times higher maximum slope (R_i) of the canopy net assimilation than that in plants grown under ambient [CO_2] (Table 2). Plants grown under elevated [CO_2] reached the plateau value (90% of maximum) of maximum canopy net assimilation rate at day 22, while for ambient [CO_2] plants this was observed at day 33 (Fig. 2A). Elevated growth [CO_2] led to a maximum canopy isoprene emission flux ($I_{max,canopy}$) of 38 µmol day^{-1}, which is 1.4 times higher than the maximum value of canopy isoprene emission rate of 27 µmol day^{-1} under ambient [CO_2] (Fig. 2B, Table 2). The relative growth rates of isoprene emission were not much different for both treatments, but elevated [CO_2] grown plants had significantly higher maximum slope (R_i) of the canopy isoprene emission rate and the inflexion point of fastest emission change rate was reached by one day earlier (14th day) (Fig. 2B, Table 2).

Table 2. Mean (± SE) parameters of canopy-scale daily net assimilation and isoprene emission rates during canopy development fitted by Eq. 1, (Modified from **Paper I**).

Net assimilation	$A_{max,canopy}$ (mmol day^{-1})	r (day^{-1})	c	t_i (day)	A_i (mmol day^{-1})	R_i (mmol day^{-2})
Ambient	16.0±0.9	0.17±0.017	9.7±2.6	12±1	5.36±0.37	1.07±0.07
Elevated	32.0±0.9*	0.25±0.035*	31±9*	12±1	11.33±0.43*	3.07±0.43*

Isoprene emission	$I_{max,canopy}$ (mmol day^{-1})	r (day^{-1})	c	t_i (day)	I_i (mmol day^{-1})	R_i (mmol day^{-2})
Ambient	27.2±1.8	0.238±0.012	39±8	15±1	9.8±0.7	2.45±0.26
Elevated	37.6±2.2*	0.220±0.014	23.2±4.5*	14±1	13.4±0.8*	3.11±0.23*

$A_{max,canopy}$ is the maximum canopy daily assimilation rate and, $I_{max,canopy}$ is the maximum canopy daily isoprene emission rate. Parameters (y_0, r, c) have the same meaning as in Table 1. Parameters for the time (t_i) and process rates (A_i or I_i) and R_i have the same meaning as in Table 1. n = 10 for all parameters, and * denotes a significant difference between the mean values at $P < 0.05$ (paired t-tests).

In the leaf scale, the trends of the developmental dynamics of leaf net assimilation rate and isoprene emission rate were changed to asymmetric curves with maxima in both [CO_2] treatments (Fig. 2 C and D). Both processes were well fitted by Eq. 2 (r^2>0.98, P<0.0001). The decreases in the fluxes beyond the maxima were assumed to be caused by increasing self-shading within the growing canopy. At the leaf level, elevated growth [CO_2] resulted in a maximum net assimilation flux of 8.7 µmol m^{-2} s^{-1} that occurred at 18th day. This rate was 1.4 times higher than that in ambient [CO_2] growth plants (6.1 µmol m^{-2} s^{-1}) on the same day (Fig. 2C). The maximum isoprene emission rate was observed on day 25 for plants grown under elevated, and on day 23 for ambient [CO_2] grown plants. At leaf level, plants grown under elevated [CO_2] released 1.3 fold less isoprene than the plants grown under ambient [CO_2] (Fig. 2D).

For both canopy and leaf scales, the model curve parameters for all processes considered strongly depended on plant growth [CO_2]. These results demonstrate that canopy scale dynamics importantly complement the leaf scale processes.

5.2. Elevated growth [CO_2] impacted leaf traits (Papers I-III)

Elevated growth [CO_2] significantly impacted leaf structure, resulting in greater leaf thickness, mesophyll thickness, chloroplast number per

cell area, starch grains inside the chloroplasts and foliar dry to fresh mass ratio (D_F), compared with those ambient [CO_2] grown plants (Fig. 3; Fig. 4). These data indicate that elevated growth [CO_2] led to more developed photosynthetic tissue.

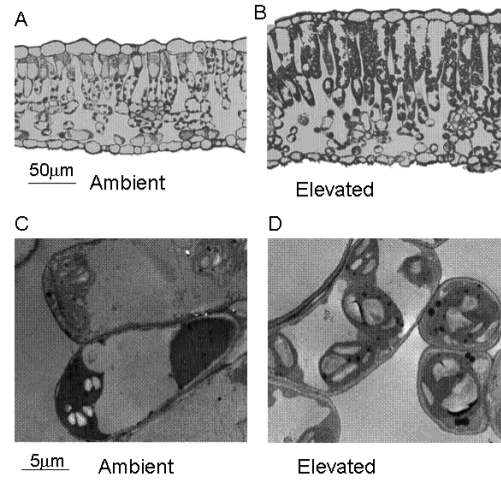


Figure 3. Representative leaf cross-sections of hybrid aspen leaves stained with Toluidine blue and viewed at 40x magnification (A and B) and leaf palisade mesophyll cell images with representative starch grain inside the chloroplasts viewed with TEM at 2100x magnification (C and D). Leaves developed under the ambient [CO_2] of 380 mmol mol^{-1} (A and C) and elevated [CO_2] of 780 mmol mol^{-1} (B and D).

Elevated growth [CO_2] significantly enhanced whole canopy leaf dry mass (M_C), leaf dry mass per unit area (M_A), leaf carbon content per leaf area (C_A), ratio of C:N ($R_{C:N}$), though the degree of stimulated was different between the two clones (Table 3). Carbon content per dry mass (C_M) was not affected by growth [CO_2], but nitrogen content per dry mass (N_M) was significantly lower for plants grown under elevated [CO_2] (Table 3). In plants of clone H200, nitrogen content per leaf area (N_A) was significantly enhanced, while for the clone H55 there was only a slight decrease (Table 3). These data suggested that elevated growth [CO_2] enhanced carbon and nitrogen uptake, but the degree of enhancement was higher for carbon than for nitrogen, and differed between the clones.

Table 3. Effects of plant growth CO_2 (ambient (380 μmol mol^{-1}) vs. elevated (780 μmol mol^{-1})) on average ± SE values of leaf morphological and chemical characteristics. (Modified from **Papers I- II**).

Trait	Clone	Treatment		*P*
		Ambient	Elevated	
Canopy leaf dry mass (M_C, g $plant^{-1}$)	H55	1.64±0.21	2.64±0.14	0.003
	H200	1.72±0.22	2.98±0.22	<0.001
Leaf dry mass per unit area (M_A, g m^{-2})	H55	28.5±1.9	35.0±1.5	0.03
	H200	28.8±0.6	38.6±0.8	<0.001
Leaf carbon content per dry mass (C_M, %)	H55*	43.29±0.33	43.53±0.29	0.6
	H200	37.4±0.6	39.2±1.2	0.07
Leaf carbon content per leaf area (C_A, g m^{-2})	H55	12.4±0.8	15.95±1.1	0.02
	H200	10.76±0.30	14.91±0.36	<0.001
Leaf nitrogen content per dry mass (N_M, %)	H55*	2.34±0.24	1.66±0.13	0.03
	H200	4.21±0.08	3.36±0.05	<0.001
Leaf nitrogen content per leaf area (N_A, g m^{-2})	H55*	0.7±0.07	0.64±0.036	0.5
	H200	1.211±0.019	1.29±0.03	0.04
Carbon to nitrogen ration in foliage ($R_{C:N}$)	H55*	19.0±1.7	26.2±2.22	0.03
	H200	8.88±0.13	11.51±0.25	<0.001

n = 8 for clone H200 and *n* = 10 for clone H55. Means (± SE) between treatments were compared by ANOVA (followed by Tukey's test). The differences among the clones were tested by univariate statistical analysis, * represent significant differences between the clones at $P < 0.05$.

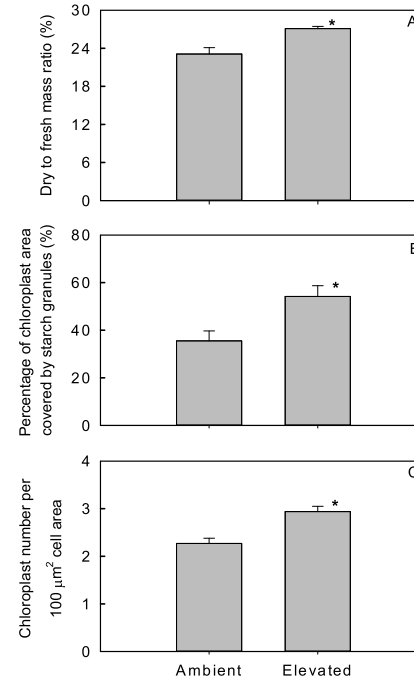


Figure 4. Effects of growth CO_2 concentration on hybrid aspen leaf dry to fresh mass ratio (%) (A), percentage chloroplast area covered by starch granules (%) (B) and chloroplast number per 100 mm^2 cell area (nchl) (C). Data are mean ± SE of four independent samples (trees). Means were compared by one way ANOVA (followed by Tukey's test) and * of bar top are significantly different at $P < 0.05$.

5.3. Modification of instantaneous [CO_2] response of photosynthesis and isoprene emission rates, DMADP pool size and isoprene synthase rate constant by growth [CO_2] (Paper II)

After 30-40 days of plants growth, instantaneous [CO_2] responses of foliage physiological traits were measured. At growth light intensity of 500 µmol m^{-2} s^{-1} and temperature of 30 °C, and measurement [CO_2] of 380 µmol mol^{-1}, net assimilation rate for plants grown under elevated [CO_2] did not differ from that in plants grown under ambient [CO_2]. However, when measured at [CO_2] of 780 µmol mol^{-1}, net assimilation rate in plants grown under elevated [CO_2] was significantly higher than that in plants grown under ambient [CO_2] (Fig. 5A). These data suggested that elevated [CO_2] grown plants had higher potential photosynthetic capacity at higher ambient [CO_2] conditions.

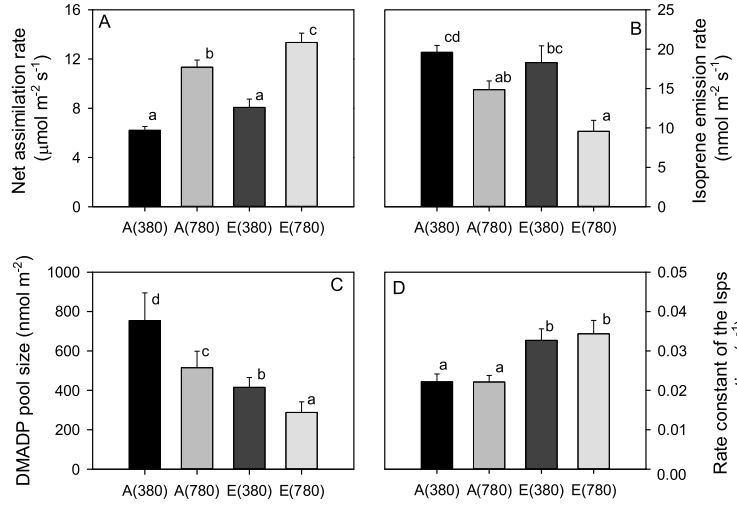


Figure 5. Effects of growth and measurement CO_2 environments on net assimilation rate (A), isoprene emission rate (B), DMADP pool size (C), and the rate constant of the IspS reaction (D) in hybrid aspen leaves. All measurements were conducted under growth leaf temperature of 30 °C, and growth light intensity of 500 mmol m^{-2} s^{-1}. A(380) and E(380) denote measurements at CO_2 concentration of 380 mmol mol^{-1} for plants grown under ambient and elevated [CO_2], and A(780) and E(780) denote measurements at CO_2 concentration of 780 mmol mol^{-1} for plants grown under ambient and elevated [CO_2]. Data are averages (± SE) of 8-10 replicate leaves. Averages with different letters are significantly different at $P < 0.05$ (ANOVA followed by Tukey's test and paired samples *t*-tests for differences among CO_2 measurement concentrations within treatments) (Reproduced from **Paper II**).

For isoprene emission, elevated growth [CO_2] only slightly decreased isoprene emission rates either at measurement [CO_2] of 380 µmol mol^{-1} or 780 µmol mol^{-1} (Fig. 5B). Either measured at [CO_2] of 380 µmol mol^{-1} or 780 µmol mol^{-1}, DMADP pool in elevated [CO_2] grown plants was significantly lower than that in ambient [CO_2] grown plants (Fig. 5C). In addition, isoprene emission rate and DMADP pool size were both influenced by measurement [CO_2] (Fig. 5C). Isoprene synthase rate constant (*K*) that characterizes the isoprene synthase (ISPS) activity, did not depend on measurement [CO_2], but growth under elevated [CO_2] resulted in greater ISPS activity (Fig. 5D).

5.4. Growth under elevated [CO_2] altered parameters of photosynthetic and isoprene emission response curves to light and CO_2 (Paper II)

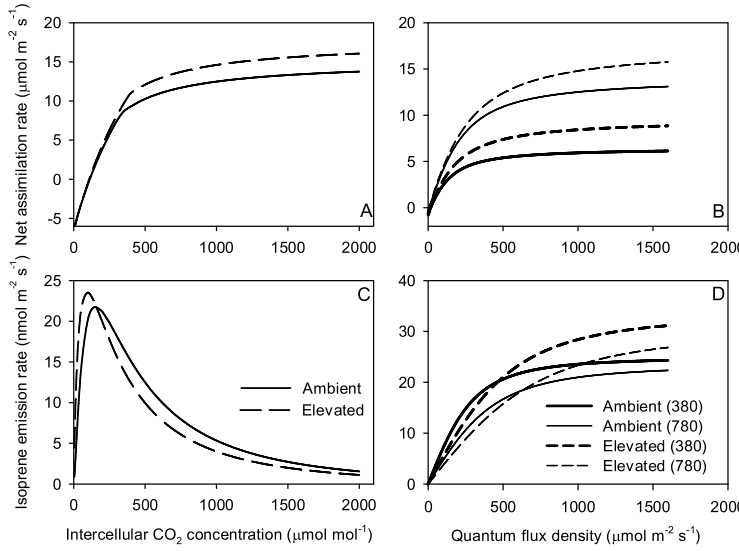


Figure 6. Simulated net assimilation (A, B) and isoprene emission rate (C, D) responses to intercellular CO_2 concentration (A, C) and to quantum flux density (B, D) in hybrid aspen plants grown under ambient and elevated CO_2 concentrations based on the mean fitted parameters (Tables 4 and 5). In the simulations, leaf temperature was set to growth leaf temperature of 30 °C, and the CO_2 responses were simulated at growth light intensity of 500 mmol m^{-2} s^{-1}, and the light responses at CO_2 concentrations of 380 mmol mol^{-1} and 780 mmol mol^{-1} (Reproduced from **Paper II**).

Growth under elevated [CO_2] altered the parameters of photosynthesis and isoprene emission rate vs. light intensity and CO_2 response curves (Fig. 6, Table 4, Table 5). Modelled response curves of intercellular CO_2 (C_i) vs. photosynthesis rate demonstrated that elevated growth [CO_2] resulted in higher maximum carboxylase activity of Rubisco (V_{cmax}) and the capacity for photosynthetic electron transport (J_{max}) (Fig. 6A), suggesting greater biochemical photosynthesis potentials in plants grown under elevated [CO_2]. Thus, no downregulation of photosynthetic characteristics was observed (Table 4A, Fig. 6A).

Table 4. Average ± SE characteristics of net assimilation (A) and isoprene emission (I) vs. intercellular CO_2 concentration (C_i) response curves: parameters are form Farquhar *et al.* (1980) photosynthesis model and modified Weibull model to fit I vs. C_i responses (e.g. Yang *et al.* 1978 with modifications outlined in **Paper II**).

Characteristic	Treatment		*P*
	Ambient	Elevated	
A: Photosynthetic characteristics			
Maximum carboxylase activity of Rubisco (V_{cmax}, mmol m^{-2} s^{-1})	39.3±2.0	44.8±1.6	0.03
Capacity for photosynthetic electron transport (J_{max}, mmol m^{-2} s^{-1})	70.1±2.7	87±6	0.02
J_{max}/V_{cmax} (mmol electrons (mmol CO_2)$^{-1}$)	1.80±0.06	1.96±0.07	0.08
Mitochondrial respiration rate (R_d, µmol m^{-2} s^{-1})	0.65±0.09	0.52±0.07	0.2
B: Isoprene emission characteristics			
Intercellular CO_2 concentration for maximum isoprene emission rate ($C_{i,max}$, µmol mol^{-1})	154.5±3.3	96±5	<0.001
Isoprene emission rate at $C_{i,max}$ ($I_{max,ci}$, nmol m^{-2} s^{-1})	21.9±2.0	28.1±3.4	0.02
Isoprene emission rate at C_i = 300 mmol mol^{-1} (I_{Ci300}, nmol m^{-2} s^{-1})	17.4±1.1	14.0±1.0	0.002
Isoprene emission rate at C_i = 500 mmol mol^{-1} (I_{Ci300}, nmol m^{-2} s^{-1})	13.2±0.9	10.0±0.8	0.009

The measurements were conducted at an incident quantum flux density of 500 µmol m^{-2} s^{-1}. Data are averages ± SE of 8-10 replicates at each treatment. Means were compared by ANOVA.

Light response curve parameters (model based on Thornley (1976) and Ögren *et al.* (1993) indicated that elevated [CO_2] grown plants had significantly higher initial quantum yield for CO_2 (Φ) and initial quantum yield for photosynthetic electron transport (Φ_J), than

those in ambient [CO_2] grown plants, either measured at [CO_2] 380 or 780 mmol mol^{-1}. Light-saturated net assimilation rate (A_{max}) in elevated [CO_2] grown plants was slightly increased (Fig. 6B, Table 5A). Furthermore, plants grown under ambient [CO_2] had a greater curvature (K) of light response at both measurement CO_2 concentrations (Table 5A). Further more, Φ_J and K mainly depended on growth [CO_2], while A_{max}, Φ and dark respiration rate (R_{dk}) were influenced by both growth and measurement [CO_2]. These data indicated interactive effects of growth and measurements [CO_2] on photosynthetic light response.

The response curve of isoprene emission rate (I) vs. C_i response is an asymmetric curve with a peak value. The shape of this response was importantly affected by plant growth CO_2 concentration (Fig. 6C). Analysis of isoprene emission rate (I) vs. C_i response curves (model modification from Guenther *et al.*, 1993, Niinemets *et al.*, 2010a) demonstrated that plants grown under elevated [CO_2] had higher maximum isoprene emission rate ($I_{Ci,max}$) and correspondingly lower $C_{i,max}$ (Table 4B, Fig. 6C).

The light-saturated isoprene emission rate ($I_{max,Q}$) was significantly higher in elevated [CO_2] grown plants under both measurement CO_2 concentrations of 380 and 780 mmol mol^{-1} (Table 5B, Fig. 6D). The "true" quantum yield of isoprene emission, α_T, did not statistically differ among the treatments at both measurement CO_2 concentrations, but higher measurements [CO_2] (780mmol mol^{-1}) obviously decreased α_T and the ratio of α_T/Φ_J (Table 5B) for both [CO_2] treatments, indicating that the higher measurement [CO_2] reduced the efficiency for the use of photosynthetic electron transport in isoprene emission.

Table 5. Average ± SE parameters of net assimilation (A) and isoprene emission (B) rate vs. light (Q) response curves in plants grown under ambient and elevated [CO_2] and measured at two different CO_2 concentrations. Light response curves of photosynthesis were fitted by a non-rectangular hyperbola (Ögren *et al.* 1993), and Isoprene emission vs. light response curves were fitted by the light response function of modified Guenther *et al.* isoprene emission model (Guenther *et al.*, 1993, Niinemets, 2010a) (Reproduced from **Paper II**).

Characteristic	**Treatment (measurement CO_2 concentration, mmol mol^{-1})**			
	Ambient (380)	**Ambient (780)**	**Elevated (380)**	**Elevated (780)**
A: Parameters of net assimilation (A) vs. light response				
Light-saturated A (A_{max}, mmol m^{-2} s^{-1})	7.1±0.9^{a}	14.5±1.4^{bc}	10.3±1.1^{ab}	17.8±1.4^{c}
Initial quantum yield for CO_2 (Φ, mol mol^{-1})	0.0441±0.0038^{a}	0.0547±0.0023^{b}	0.0560±0.0019^{b}	0.0633±0.0030^{c}
Initial quantum yield for photosynthetic electron transport (Φ_J, mol mol^{-1})	0.272±0.019^{a}	0.271±0.011^{a}	0.341±0.012^{b}	0.316±0.015^{b}
Dark respiration rate (R_{dk}, mmol m^{-2} s^{-1})	0.63±0.17^{bc}	0.38±0.18^{a}	0.84±0.17^{c}	0.52±0.16^{ab}
Curvature (k)	0.67±0.05^{b}	0.64±0.07^{b}	0.49±0.05^{a}	0.47±0.06^{a}
B: Parameters of isoprene emission (I) vs. light response				
Light-saturated I ($I_{max,Q}$, nmol m^{-2} s^{-1})	25.8±2.0^{a}	22.6±1.7^{a}	38.5±3.2^{b}	35.8±2.9^{b}
I at Q = 1000 (I_{Q1000}, nmol m^{-2} s^{-1})	23.8±2.3^{b}	19.8±1.7^{a}	30.0±2.6^{c}	24.2±3.0^{ab}
$I_{max,Q}/I_{Q1000}$ (C_L)	1.101±0.021^{a}	1.31±0.06^{bc}	1.152±0.019^{ab}	1.40±0.07^{c}
Apparent quantum yield (α, m^2 s nmol^{-1})	2.82±0. 21^{c}	2.04±0. 26^{b}	1.57±0. 14^{b}	1.09±0. 11^{a}
True quantum yield (α_T, mmol mol^{-1})	0.063±0.005^{c}	0.0372±0.0034^{ab}	0.050±0.005^{bc}	0.02922±0.0046^{a}
Ratio α_T/Φ_J (mmol mol^{-1})	0.259±0.029^{c}	0.154±0.017^{b}	0.149±0.019^{b}	0.099±0.011^{a}

Data are means ± SE of 8-10 independent replicates. The means followed by different letters are significantly different at $P < 0.05$ (ANOVA followed by Tukey's test for treatment differences and paired samples t-tests for differences among measurement CO_2 concentrations within treatment).

5.5. Elevated growth [CO_2] results in higher stress resistance coupled with higher isoprene emission rate (Paper III)

Long-term elevated [CO_2] treatments altered foliage photosynthetic and isoprene emission responses to instantaneous variations in [CO_2] and light, suggesting that acclimation to elevated [CO_2] may be associated with modified responses to extreme light and [CO_2] conditions. Studies with extreme temperatures further indicated that growth [CO_2] alters heat stress resistance. With temperature increase from 30 ^oC to 50 ^oC, net assimilation rate was completely inhibited, while isoprene emission rate increased among treatments (Fig. 7A, B, C, D), confirming the previous investigations that isoprene emission rate can be uncoupled from photosynthesis under stress conditions, and that stress conditions can lead to higher carbon losses due to isoprene emission (Fig. 7E, F). However, plants grown under elevated [CO_2] maintained a greater net assimilation rate at the same high temperature than those grown under ambient [CO_2], particular under measurements [CO_2] of 780 mmol mol^{-1} and strong light intensity of 2000 mmol m^{-2} s^{-1}. With temperature enhancement, both the reduction of net assimilation and increase of isoprene emission rates tended to be higher, but plants grown under elevated [CO_2] and measured under [CO_2] of 780 mmol mol^{-1}, had the lowest reduction of net assimilation rate accompanied with the highest increase of isoprene emission rate at given temperature, particularly if measured under strong light intensity of 2000 mmol m^{-2} s^{-1} (Fig. 8).

A linear relationship between the reduction in net assimilation rate and the increase in isoprene emission rate was observed (Fig. 9). Co-variation analyses (ANCOVA) indicated presence of significant interactions among the measurement and growth [CO_2]. Under moderate light, the slope varied as Ambient (380) > Elevated (380) > Ambient (780) > Elevated (780), while under high light the slope ranked as Ambient (380) > Ambient (780) > Elevated (380)> Elevated (780) (Fig. 9). These data indicate that under both high light and heat stress, the suppression of photosynthesis was less in elevated [CO_2] grown plants, especially under higher measurement [CO_2] of 780 mmol mol^{-1}.

An interesting finding was that the inhibition of isoprene emissions by high measurement [CO_2] disappeared under heat stress, and isoprene

emission rate was mainly related to plants growth [CO_2] (Fig. 7C, D). This may reflect differences in the regulation of isoprene formation under stress, especially by substrate supply. We propose a hypothesis on isoprene regulation during stress in the **sections of 6.6**.

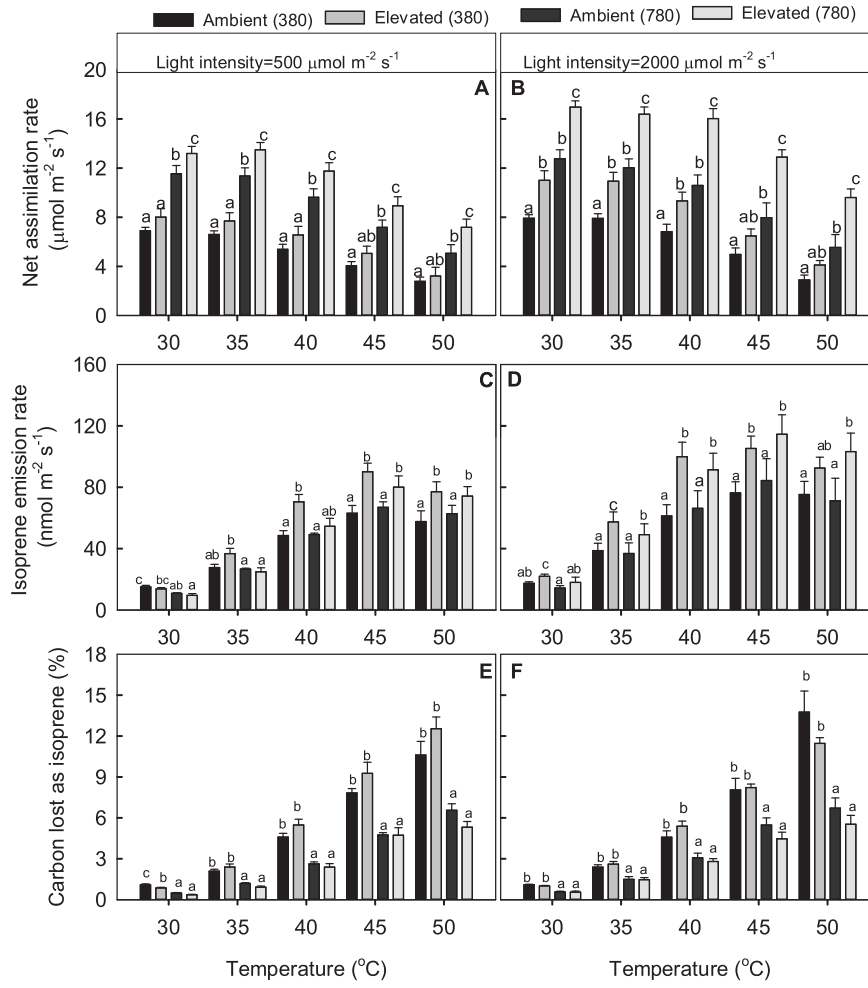


Figure 7. Responses of net assimilation rate (A, B), isoprene emission rate (C, D) and carbon lost as isoprene (E,F) to heat stress in hybrid aspen leaves under different growth (ambient [CO_2] of 380 mmol mol^{-1} and elevated [CO_2] of 780 mmol mol^{-1}) and measurement CO_2 conditions (380 vs. 780 mmol mol^{-1}). Averages with different letters are significantly different at $P < 0.05$ (ANOVA followed by Tukey's test and paired samples *t*-tests for differences among CO_2 measurement concentrations within treatments) (Modified from **Paper III**).

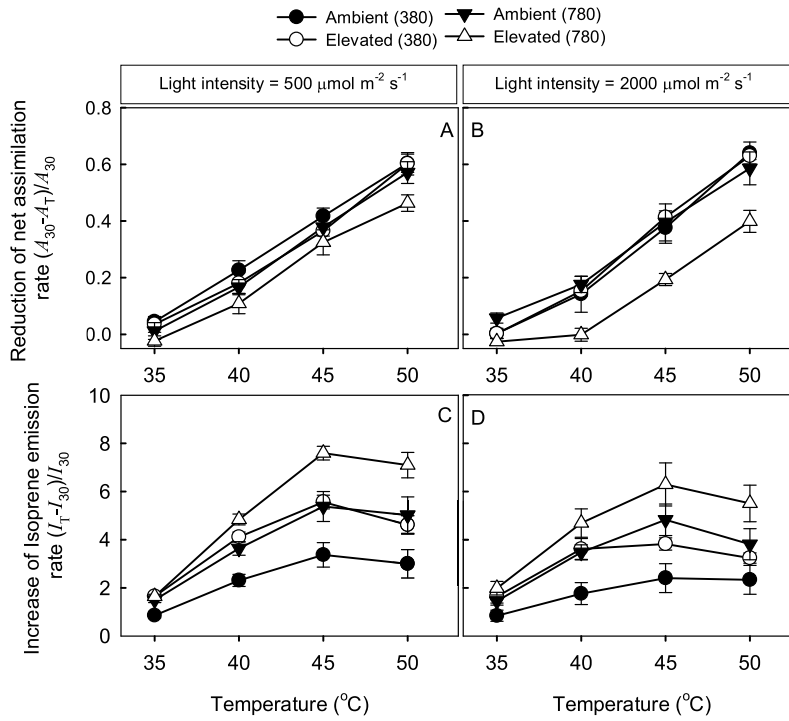


Figure 8. The dependencies of the relative decrease in net assimilation rate (A, B) and the relative increase in isoprene emission rate (C, D) on leaf temperature in hybrid aspen. The relative decrease in net assimilation rate was calculated as $(A_{30}\text{-}A_T)/A_{30}$. The relative increase in isoprene emission rate was calculated as $(I_{30}\text{-}I_T)/I_{30}$. A_{30} and A_T represent the net assimilation rate at the temperature of 30 °C and at given temperature T °C (T = 35, 40, 45, 50 °C), I_{30} and I_T represent the isoprene emission rate at the temperature of 30 °C and T °C (T = 35, 40, 45, 50 °C). Data are averages (± SE) of 8-10 replicate leaves (Modified from **Paper III**).

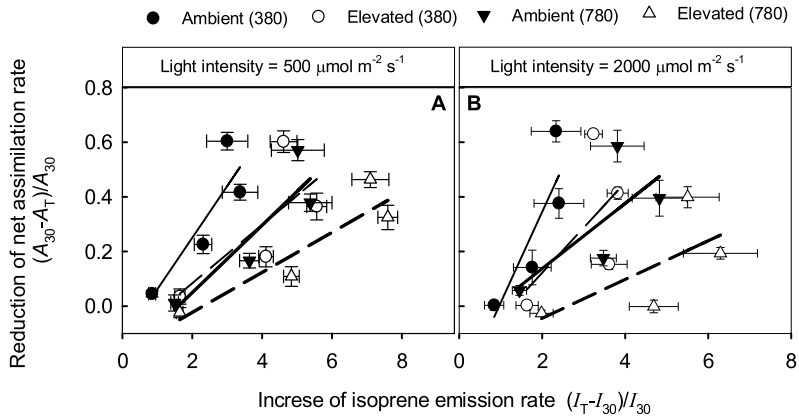


Figure 9. Correlations of the relative decrease of net assimilation rate with relative increase of isoprene emission rate during the heat stress under different growth (ambient [CO_2] of 380 mmol mol^{-1} and elevated of 780 mmol mol^{-1}) and measurement CO_2 conditions (380 vs. 780 mmol mol^{-1}) and measured under light intensity of 500 mmol m^{-2} s^{-1} (A) 2000 mmol m^{-2} s^{-1} (B). Data were fitted by linear regressions. For light intensity of 500 mmol m^{-2} s^{-1}, black solid line: Ambient (380) at(r^2 = 0.76 and P < 0.01); black dashed line: Elevated (380) (r^2 = 0.53 and P < 0.01); solid grey line: Ambient (780) (r^2 = 0.79 and P < 0. 01); dashed grey line: Elevated (780) (r^2 = 0.84 and P < 0.001). For light intensity of 2000 mmol m^{-2} s^{-1}, black solid line: Ambient (380) (r^2 = 0.74 and P < 0. 01); black dashed line: for Elevated (380) (r^2 = 0.35 and P = 0. 1); solid grey line: Ambient (780) (r^2 = 0.59 and P < 0.01); dashed grey line: Elevated (780) (r^2 = 0.45 and P = 0.05) (**Paper III**).

5.6. Control of isoprene emission shared between environment temperature and foliage senescence during autumn (Paper IV)

Photosynthetic rate, isoprene emission and DMADP pool decreased rapidly with reduction of growth environment temperature and foliage senescence, though a certain recovery was observed during episodes of temperature increase (Fig. 10). Leaf nitrogen content decreased slowly during the initial phase of leaf senescence, and was rapidly reduced during later phases (Fig. 10). The correlation analysis indicated that changes in foliage nitrogen content were closely related to reductions in net photosynthetic capacity and isoprene emission rate (Fig. 11). This study demonstrated that the capacity for isoprene emission can adjust to environmental conditions in senescing leaves as well, but the responsiveness is low compared with mid-season and is also affected by frost stress episodes in senescing leaves (Fig. 12).

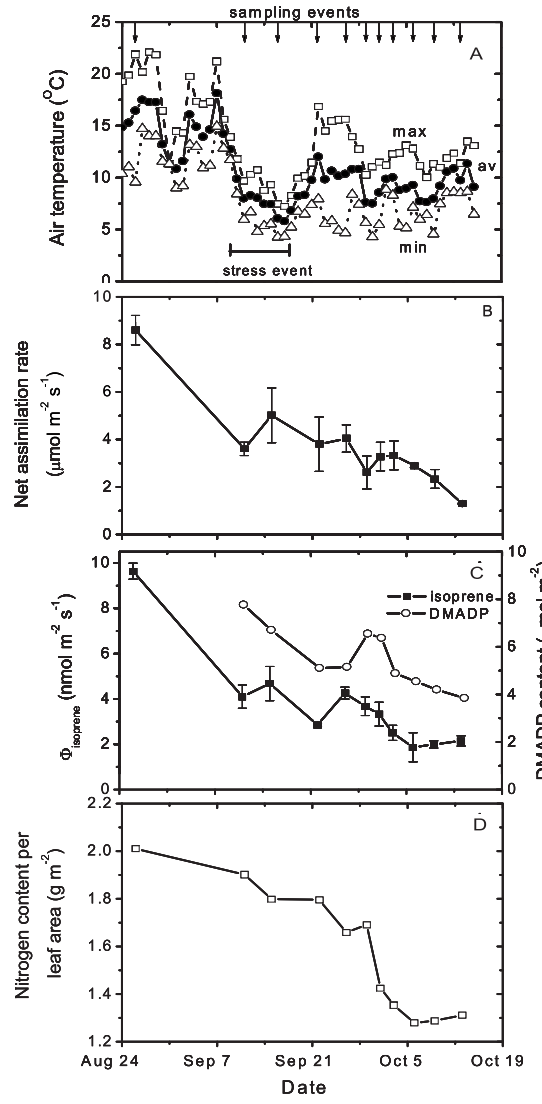


Figure 10. Time-dependent variations in daily minimum, maximum and average air temperature at Tartu (A), and in net assimilation rate (B), isoprene emission rate and DMADP pool size (C), and leaf nitrogen content per area (D), from late summer to leaf fall in European aspen (*Populus tremula*) trees. Data in B-D are averages ± SE of three independent samples. Foliage gas-exchange measurements were conducted at an ambient CO_2 concentration of 380 ± 20 mmol mol^{-1}, leaf temperature of 30 ± 1 °C and incident quantum flux density of 600 ± 100 mmol m^{-2} s^{-1}. (Modified from **Paper IV**).

Figure 11. Dependencies of leaf isoprene emission (A) and net assimilation (B) rates on leaf nitrogen content in European aspen (*Populus tremula*) trees from late summer through leaf senescence. Data were fitted by either non-linear regressions in the form of $y = ax^b$ (all data pooled) or by linear regressions (fitting without the late-summer measurements). Data in A were separately fitted using all data pooled (dashed line) and with the late summer estimate removed (solid line). Error bars show ±SE (Reproduced from **Paper IV**).

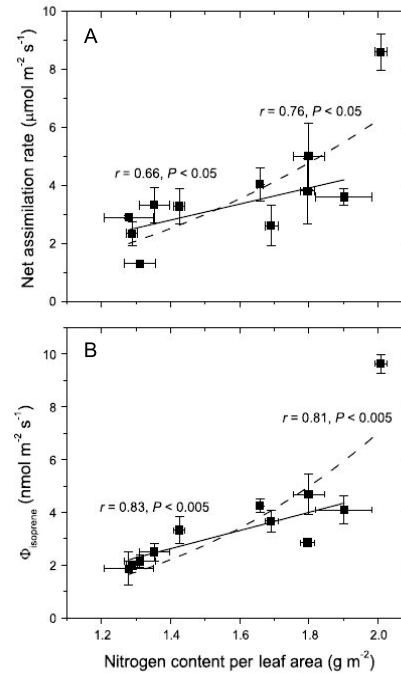
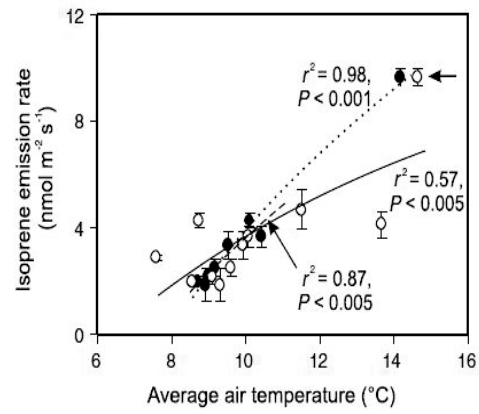


Figure 12. Correlations of isoprene emission rate with average temperature preceding the measurements in *P. tremula.* Data were fitted by non-linear regressions in the form of y = aLog(x)+b. Open circles and fitted solid line were based on data pool (from late summer to leaves falling down); filled circles in and fitted dotted line were for whole data but removed corresponding to stress period data; and fitted dashed line was based on only the data after stress (Reproduced from **Paper IV**).



6. DISCUSSION

In this thesis, we investigated foliage structure and anatomy, photosynthetic and isoprene emission characteristics, and DMADP pool size and ISPS activity, to gain insight into plant acclimation to long-term elevated [CO_2]. We tested the hypothesis that the canopy isoprene emission of hybrid aspen grown under elevated [CO_2] is increased even though an instantaneous effect of elevated [CO_2] lowers isoprene emission at the level of individual leaves. We found that long-term elevated [CO_2] treatment enhanced isoprene emission capacity, photosynthesis capacity, which are associated with changed foliar ontogenetic characteristics, DMADP pool size and isoprene synthase activity. In addition, elevated growth [CO_2] enhanced leaf isoprene emission capacity and stress resistance, implying that isoprene emission may be enhanced by these potential factors in future elevated atmospheric [CO_2]. We further studied isoprene emission characteristics during the foliage senescence, to test environmental temperature and endogenous, senescence-induced self-regulation on isoprene and methanol emissions. Here I discuss in detail the specific results of the Thesis.

6.1. Effects of elevated [CO_2] on isoprene emission in different scales (Paper I)

Many previous studies have predicted that the continuous rise in atmospheric [CO_2] affects global isoprene emissions and in turn can affect climate change in future (Sanderson *et al.*, 2003, Wilkinson *et al.*, 2009, Young *et al.*, 2009). Up to now, understanding elevated [CO_2] effects on plant isoprene emissions is far from conclusive because of uncertainties in isoprene emission capacities in plant species, alteration of isoprene emissions by [CO_2], and changes in coverage of isoprene emitting species, their biomass as well as due to scaling problems (Pacifico *et al.*, 2009). Available empirical and semi-mechanistic models based on the light and temperature responses analogous to photosynthesis describe isoprene emissions under current ambient CO_2 concentrations relatively well for non-stressed plants in leaf level (Arneth *et al.*, 2007, Guenther *et al.*, 1993, Niinemets *et al.*, 2010c). These models have predicted that global isoprene emissions will increase due to elevated [CO_2] that stimulate greater vegetation productivity, especially when combined with warming that enhances temperature-dependent isoprene emissions (Arneth *et al.*,

2008a, Guenther *et al.*, 1995, Wiedinmyer *et al.*, 2006). However, when inhibition of isoprene emissions by elevated [CO_2] at leaf level observed in several studies (Pegoraro *et al.*, 2004, Rasulov *et al.*, 2009b, Rosenstiel *et al.*, 2003) was considered, it was projected that isoprene emissions will be reduced in future climates (Arneth *et al.*, 2007, Heald *et al.*, 2009, Monson *et al.*, 2007, Young *et al.*, 2009).

In this thesis, we investigated dynamics in development of leaf area, net assimilation and isoprene emission rates from the start of canopy development to maturation under ambient and elevated growth [CO_2]. Our data indicated that the effects of elevated [CO_2] on isoprene emission were differently expressed at different scales (Fig. 2B, D). Elevated growth [CO_2] enhanced canopy leaf area development rate, net assimilation rate, and isoprene emission rate, although different processes were affected to a different degree. Although elevated growth [CO_2] inhibited isoprene emission rate at leaf scale, in accordance with previous studies (e.g. Monson *et al.*, 2007, Possell *et al.*, 2011, Rosenstiel *et al.*, 2003), enhanced leaf area production compensated for or exceeded the leaf-level isoprene emission inhibition such that canopy-level isoprene emission was enhanced by elevated growth [CO_2] under unstressed conditions. With canopy growth, the light distribution inside of canopy became an important factor affect isoprene emission rate in leaf scale, but greater foliage area still dominated whole canopy isoprene emissions. Thus, this study demonstrated that the canopy scale dynamics importantly complements the leaf scale processes, and it is important to consider growth processes in modelling canopy scale isoprene emissions.

6.2. Modulation photosynthetic capacity and isoprene emission by growth [CO_2] (Paper II)

Previous studies have indicated that the effects of elevated growth [CO_2] on plants growth are associated with many aspects of plant physiology (Ainsworth *et al.*, 2005, Jablonski *et al.*, 2002, Leakey *et al.*, 2009). Morphological up-regulation due to higher leaf thickness and more structured mesophyll under elevated [CO_2] has been observed (e.g., Oksanen *et al.*, 2001, Sims *et al.*, 1998, Miyazawa *et al.*, 2011). However, elevated [CO_2] can also result in biochemical down-regulation of photosynthesis owing to enhanced carbon supply, especially when nutrient supply is limited. Such imbalances are reflected in extensive

accumulation of starch and soluble carbohydrates, and in stronger N limitation for construction of photosynthetic machinery, leading to reduced foliage N content and decreased photosynthetic capacity due to lower maximum carboxylation activity of Rubisco (V_{cmax}) and maximum electron transport capacity (J_{max}) (Johnson, 2006, Long *et al.*, 2004, Luo *et al.*, 1998, Nowak *et al.*, 2004). These contrasting aspects have received surprisingly little attention in isoprene emission studies.

In this study (Paper I and II), elevated [CO_2] grown plants had significant lower N content per dry mass (Table 1), but N content per leaf area even increased in hybrid aspen clone H200 and was not affected by elevated [CO_2] in the other clone H55 (Table 1). This suggests that the uptake of carbon was somewhat in excess to nitrogen availability, although N deficiency clearly was not severe, and there was no biochemical downregulation of photosynthesis occurred. In fact, the plants grown under elevated [CO_2] had higher photosynthetic capacity with higher V_{cmax} and J_{max}, and greater quantum yield of photosynthesis (Φ) and photosynthetic electron transport (Φ_J) (Table 4A). This evidence supports previous findings that high supply of nutrients can reduce or remove down-regulation to maintain higher photosynthetic machinery and capacity, especially by morphological up-regulation (Ainsworth *et al.*, 2005, Leakey *et al.*, 2009, Luo *et al.*, 1998).

Under the plant growth conditions of moderately high light of 500 µmol m^{-2} s^{-1} and leaf temperature of 30 °C, growth [CO_2] did not affect significantly isoprene emission rate (Fig. 5B). However, plants grown under elevated [CO_2] had higher isoprene emission rate at optimum CO_2 concentration (Table 4B, Fig. 6C) and at saturation light (Table 5B, Fig. 6D), indicating that elevated growth [CO_2] enhanced isoprene emission capacity. Compared with previous studies, the reduction of isoprene emission under elevated growth [CO_2] has been observed only when elevated [CO_2] also resulted in down-regulation in photosynthetic capacity e.g. (Monson *et al.*, 2007, Possell *et al.*, 2005, Wilkinson *et al.*, 2009). On the contrary, the study of Possell et al. (2004) found that fertilization reduced the depression of isoprene emissions under elevated [CO_2]. Thus, it is possible that limiting nutrients such as N for protein synthesis, ultimately drive modifications in enzymatic capacities for both photosynthesis and isoprene emission.

Isoprene is formed in chloroplasts by isoprene synthase from its immediate precursor DMADP, and thus, the availability of DMADP and ISPS activity are important intrinsic factors determining isoprene emission rate. In this study, elevation of growth [CO_2] resulted in reduced DMADP pool size and higher ISPS activity. DMADP pool size is influenced by instantaneously changes in [CO_2], while ISPS activity is believed not to be influenced by instantaneously changes in [CO_2] (Rasulov et al. 2009, 2010). Thus, the response of isoprene emissions to instantaneous changes in [CO_2] is mainly influenced by the pool size of DMADP, while acclimation to growth [CO_2] may involve both changes in DMADP pool size and ISPS activity. So far, few studies have analyzed the effects of changes in DMADP pool size and isoprene synthase activity on isoprene emissions in plants grown under different CO_2 concentrations. Calfapietra *et al* (2008) demonstrated reduced DMADP content in elevated-[CO_2]-grown *P. tremuloides*, but similar isoprene emission rate, suggesting enhancement of isoprene synthase activity, concurring with our observations in hybrid aspen. In the studies of Scholefield (2004) in *Phragmites australis* and Possell *et al.*(2011) in *Acacia nigrescens*, both reported that reduced isoprene emission rate was associated with reduced isoprene synthase activity in plants grown under elevated [CO_2]. However, the down-regulation of photosynthetic capacity was also observed in these studies, possibly reflecting nutrient limitations under elevated [CO_2].

Taken together, our data suggest that elevated growth [CO_2] enhanced photosynthetic capacity, but did not affect isoprene emission rate when measured at the same conditions. Elevated growth [CO_2] decreased DMADP pool size and increased ISPS activity, explaining the constancy of the isoprene emission rate. Thus, decreased DMADP pool size was partly compensated by increased ISPS.

6.3. Growth [CO_2], stress resistance and isoprene emission (Paper III)

Heat stress reduced net assimilation rate and increased isoprene emission rate, but the plants grown under elevated [CO_2] had more resistant photosynthesis with higher isoprene emission rate (Fig. 7, Fig. 8). This indirectly supports the hypothesis of the protective role of isoprene under heat stress (Sharkey *et al.*, 2008, Vickers *et al.*, 2009). Furthermore,

inhibition of isoprene emissions by instantaneous rise of [CO_2] was lost during the heat stress; isoprene emission was enhanced more strongly in plants grown under elevated [CO_2] (Fig. 7B, D), suggesting that isoprene emission in heat-stressed leaves is dominated by the long-term acclimation to growth [CO_2] environment and is not altered by instantaneous [CO_2] elevation. Elevated [CO_2] grown plants had greater starch grains inside the chloroplasts and higher soluble carbohydrate contents (Fig. 4A, B). These soluble carbohydrates together with mobilization of stored carbon sources may provide enhanced carbon flow to isoprene formation when photosynthesis is depressed by high temperature. This may explain why elevated [CO_2] grown plants with larger carbon reserves had greater isoprene emission rate and stronger thermal tolerance.

6.4. Control on isoprene emission is shared between temperature environment and foliage senescence during autumn (Paper IV)

Previous studies have revealed that isoprene emission depends on leaf developmental stage and age (Centritto *et al.*, 2004, Ekberg *et al.*, 2009, Kuzma *et al.*, 1993). During leaf senescence, the majority of soluble and membrane proteins are degraded for N resorption and cellular and subcellular structures, mitochondria and chloroplasts gradually collapse (Beers *et al.*, 2001, Hopkins *et al.*, 2007, Keskitalo *et al.*, 2005). In this study, the rapid reduction in isoprene emission rate was paralleled by decreases in foliage N content and net assimilation rate from mid-August to foliage fall. The reductions in net assimilation rate, isoprene emission rate and DMADP pool were accompanied by decreases in foliage nitrogen content, indicating that leaf physiological activities decreased in parallel with N resorption (Fig. 10, 11). At the last senescence stage, photosynthetic activity had been reduced to a greater degree than isoprene emission (Fig. 11). This is in accordance with the hypothesis that maintenance of isoprene emission helps to protect the plants against the oxidative stress that can become particularly significant during degradation of cellular components (Vickers *et al.*, 2009).

6.5. Modification of isoprene emissions and plant physiology by instantaneous and growth [CO_2] (Papers I-III)

In this study, the response of isoprene to instantaneous CO_2 concentration was characterized by an asymmetric curve with an optimum at low [CO_2], closed to the CO_2 compensation point of photosynthesis, below which isoprene emission decreased rapidly and above which isoprene emissions decreased relatively slowly (Fig. 6C), confirming previous investigations (e.g., Rasulov *et al.* 2009a). A double carboxylation scheme has been proposed, according to which RuBP carboxylase (Rubisco) in chloroplasts and PEP carboxylase in cytosol control CO_2 carboxylation in different compartments, thereby affect the substrate supply for DXP pathway (Sanadze, 2010, Monson *et al.*, 2009). When C_i is close to photosynthetic compensation point, photosynthesis is strongly limited. Although photorespiration can partly substitute CO_2 as electron sink, still RuBP carboxylation and oxygenation and photosynthetic electron transport and photophosphorylation are not well coordinated. This can result in temporal excess of ATP. Therefore, the highest isoprene emission rate at C_i close CO_2 photosynthetic compensation point might rely on this excess ATP that is consumed in DXP pathway. When C_i is above the compensation point, more ATP can be used for CO_2 fixation, resulting in the drop of ATP concentration and reduction of DMADP synthesis through DXP pathway, consequently reducing the rate of isoprene formation (Loreto *et al.*, 1993, Rasulov *et al.*, 2009b).

Light responses of photosynthesis indicated that elevated [CO_2] grown plants had significantly higher initial quantum yield for CO_2 (Φ) and initial quantum yield for photosynthetic electron transport (Φ_J), than those in ambient [CO_2] grown plants (Table 5). These results imply that elevated [CO_2] grown plants had higher light use efficiency than those grown under ambient [CO_2]. However, the light responses of isoprene emission indicated that elevated [CO_2] grown plants had lower α_T and the ratio of α_T/Φ_J. This result implied grown elevated [CO_2] plant had higher light efficiency for photosynthesis but reduced efficiency for use of photosynthetic electron transport in isoprene emission. This result is consistent with previous observation that leaves formed in elevated [CO_2] maintain higher PSII efficiency (F_v/F_m) and lower photorespiration at higher temperatures than ambient [CO_2] grown leaves (Huang *et al.*, 2007, Taub *et al.*, 2000). Furthermore, this result is consistent with the

observation of lower DMADP under moderately high light in elevated [CO_2] grown plants, reflecting overall lower rate of increase of DMADP pool size with light level. However, elevated [CO_2] grown plants had higher maximum isoprene emission rate, $I_{max,c}$, at saturating light (Fig. 6C). Thus, as DMADP pool size was increasing with increasing light level, greater ISPS activity allowed elevated [CO_2] grown plants to achieve greater isoprene emission rates.

Thus, these data collectively indicated that long-term elevated [CO_2] treatment resulted in both the enhanced photosynthetic and isoprene emission capacities, but also importantly altered the response curve shapes. These results have important implications for modelling isoprene emissions.

6.6. Regulation of isoprene emissions under heat stress (Papers II-III)

Evidence from labelling experiments has confirmed that under non-stressed conditions carbon skeletons for isoprene biosynthesis mainly come from recent CO_2 assimilation (Affek *et al.*, 2002, Lichtenthaler, 1999, Trowbridge *et al.*, 2012). However, under stress or when photosynthesis is inhibited by reduced atmospheric CO_2, stored or older carbon sources become involved in isoprene biosynthesis (Brilli *et al.*, 2007, Funk *et al.*, 2004, Trowbridge *et al.*, 2012). There are several situations when isoprene emissions are uncoupled from photosynthesis. One characteristic situation is when isoprene emission is inhibited by instantaneous elevation of [CO_2], while photosynthesis is enhanced (Centritto *et al.*, 2004, Pegoraro *et al.*, 2004, Rosenstiel *et al.*, 2003) as was also observed in our study under leaf temperature of 30 ^{o}C and moderate light intensity of 500 µmol m^{-2} s^{-1} (Fig. 7A, B). Differently from such uncoupling, uncoupling during stresses such as heat and drought and under limited CO_2 supply, is associated with major reduction in photosynthesis, but increased isoprene emission (Brilli *et al.*, 2007, Funk *et al.*, 2004, Trowbridge *et al.*, 2012). These two different situations when isoprene emissions are uncoupled from photosynthesis reflect differences in regulation of photosynthesis and isoprene biosynthesis, and might be associated with the role of isoprene in protection from stress. In this study, a particularly interesting situation was when heat stress (or heat stress and strong light) co-occurred with instantaneous increase in

[CO_2]. Under such conditions, the inhibition of isoprene emission by elevated [CO_2] disappeared, and isoprene emission rate was correlated with growth environmental [CO_2]. It is pertinent to ask what can be responsible for such a pattern?

Previous study proposed that isoprene emission is mainly regulated by its precursor DMADP pool size that depends on ATP supply and/or transport of pyruvate precursors, PEP, from cytosol (Cinege *et al.*, 2009, Rasulov *et al.*, 2009b, Vickers *et al.*, 2010, Lehning *et al.*, 1999, Trowbridge et al. 2012). Resent evidence has confirmed that isoprene emission can protect the photosynthetic systems by multiple ways, such as by removing ROS, and maintaining thylakoid membrane stability (Behnke *et al.*, 2010, Velikova *et al.*, 2005, Velikova *et al.*, 2011). Here, based on the isoprene physiological function, we hypothesise the possible regulation of flow of carbon sources to DMADP and isoprene formation between chloroplasts and cytosol under stress.

First, isoprene is a protective gas, and its emission is advantageous in protecting photosynthetic apparatus (Sharkey *et al.*, 2008). As lipophilic, highly volatile and reactive small molecule, isoprene has the virtues of fast solubilization in membranes, stabilizing lipid–lipid, lipid–protein, or protein–protein interactions, and rapid reaction, thereby efficiently scavenging reactive oxygen species (Velikova *et al.*, 2011, Vickers *et al.*, 2009). Thus, its emission from plants is an evolutionary adaptation. Studies based on phylogenetic relationships among clades of vegetation have revealed that isoprene emission has appeared and been lost many times independently during the evolution of plants, and the trait of isoprene emission has been selected during evolutionary radiation into different environments (Harley *et al.*, 1999, Monson *et al.*, 2012, Sharkey *et al.*, 2001b).

Second, under stress, DMADP flow to isoprene biosynthesis has priority over flows to pigments and higher isoprenoids in DXP pathway. Isoprene is the first product in DXP pathway catalyzed by isoprene synthase, other isoprenoids like monoterpenoids, phytol, carotenoids and plastoquinone are also formed by this way. It has also been reported that xanthophylls, tocopherols (vitamin E) and carotenoids play an important role in maintaining the integrity of the photosynthetic membranes under oxidative stress (Loreto *et al.*, 2010, Singsaas *et al.*, 1997, Velikova *et*

al., 2011, Vickers *et al.*, 2009). In fact, carotenoids and tocopherols (vitamin E) involve large number of prenyl units. Thus, formation other polyterpenes needs more energy, implying that isoprene could be economically advantageous as a small and rapidly moving molecule avoiding photosynthesis damage under stress. The study of Peñuelas *et al.* (2005) demonstrates that α-tocopherol and β-carotene consumption increased after enhancement of isoprene emission rate during heat stress. This seems indirectly support the suggestion that isoprene formation has priority over synthesis of other isoprenoids under rapidly evolving stress.

Third, under stress when photosynthesis is suppressed, isoprene biosynthesis will mobilize all available carbon sources, like old or temporary stored photosynthates, and initiate or enhance other metabolic pathway, like oxidative pentose phosphate pathway (PPP), which collectively result in enhanced supply of DMADP. Plants grown under elevated [CO_2] had more developed mesophyll tissue with greater starch grains and higher soluble sugar content. Thus, greater old or temporarily stored photosynthates may be mobilized and enter into other metabolic pathway, like oxidative pentose phosphate pathway, which produce metabolites and NADPH for isoprene formation, when photosynthesis is inhibited under stress. Such metabolic "support" of isoprene synthesis under stress is clearly needed to allows isoprene to operate as a protective compound under conditions when photosynthesis is impaired.

6.7. Implications of [CO_2] elevation on modelling isoprene emissions: instantaneous vs. acclimation responses (Papers I-III)

With atmospheric CO_2 concentration continuously rising and global warming, there are different opinions on how isoprene emissions change in future conditions. These uncertainties are in particular associated with uncertainties of how elevated [CO_2] affects isoprene emissions. In this study, our data demonstrated that the effects of elevated [CO_2] on isoprene emission were different at different functional scale and under different measurements conditions. Under non-stressed conditions, elevated growth [CO_2] inhibited isoprene emission rate under leaf scale, while canopy scale isoprene emission rate under elevated [CO_2] significantly exceeded that under current ambient [CO_2]. The reason was that elevated growth [CO_2] stimulated greater canopy leaf area formation that overcompensated the biochemical constraints on the isoprene synthesis

pathway at leaf scale (Fig. 1 and Fig. 2). Thus, different investigation scale is one of the uncertainties which influence the reliability of isoprene emission estimations under future rising atmospheric CO_2. To conduct regional or global scale estimations of isoprene emissions, canopy scale data may be useful because they are more robust than assessments for individual leaves. In particular, because variations among individual leaves might lead to large uncertainties.

Long-term elevated growth [CO_2] modulated plant growth and physiological processes in multiple levels, including canopy growth rate (Fig. 2, Table 1), foliar traits (Fig. 3 and 4, Table 3), and basic characteristics of isoprene emission rate and capacity (Fig. 5, Table 4, 5), stress responsiveness (Fig. 7). Plants benefited from elevated CO_2 by increased thermotolerance, which was coupled with enhanced isoprene emission capacity and rate under high temperature. Despite the plants grown under elevated [CO_2] had lower isoprene emission rate under moderate temperature conditions, this resulted from reduced DMADP supply. In fact, elevated [CO_2] grown plants had greater ISPS activity, which resulted in enhanced isoprene emission rate both under high light as well as under heat stress. It is predicted that rising atmospheric [CO_2] will be accompanied with global warming and enhanced evaporation, leading to more severe drought and higher temperatures in summer (Walther *et al.*, 2002). Thus, at global scale, isoprene emission amounts may be enhanced even more in future higher atmospheric [CO_2] climates.

7. CONCLUSIONS

This thesis provides information on modifications of isoprene emissions in plants grown under different atmospheric [CO_2] through bud burst to canopy maturation and during foliage senescence in plants grown under current ambient [CO_2]. Dynamics of canopy growth and net assimilation and isoprene emission rates in hybrid aspen grown under current ambient and elevated [CO_2] were studied. These studies revealed that the effects of elevated [CO_2] on isoprene emission were different between canopy and leaf level. We obtained new information on how elevated growth [CO_2] alters leaf traits and observed enhanced photosynthetic and isoprene emission capacities, although enhancement of isoprene emission capacity did not increase isoprene emission rate under moderate environmental conditions at leaf scale. However, at canopy scale the emission was strongly enhanced by elevated growth [CO_2]. Furthermore, we found that elevated growth [CO_2] improved hybrid aspen thermotolerance coupled with higher isoprene emission rate. We provided information that isoprene emission characteristics in European aspen were associated with environmental temperature and foliage endogenous, senescence-induced self-regulation during the natural senescence. Based on isoprene physiological functions and its emission responses to elevated [CO_2] and heat stress, we hypothesized that isoprene formation under stress when isoprene emission is impaired from photosynthesis is possibly regulated by the controls of the chloroplastic and cytosolic flows of carbon to DMADP. According to the results of this thesis, I draw following general conclusions:

- At the canopy scale, isoprene emission rate in elevated [CO_2] grown plants significantly exceeded that in plants developed under current ambient [CO_2]. The main reason was that elevated [CO_2] stimulated the growth of canopy leaf area, and this more than compensated for isoprene emission inhibition at leaf level. Thus, different investigation scale is one of the important uncertainties that influences the reliability of estimations of isoprene emission responses to elevated [CO_2]. To conduct regional or global scale estimations of isoprene emissions, canopy scale data may be useful because they have higher robustness against variations among individual leaves that might lead to large uncertainties.

- Growth [CO_2] strongly impacted leaf traits. Elevated growth [CO_2] resulted in significantly greater leaf thickness, mesophyll thickness, chloroplast number per cell area, starch grains inside the chloroplasts and cytosol soluble sugar concentration. This indicates that elevated [CO_2] led to more developed photosynthetic tissue and higher accumulation of photosynthates compared with those traits in ambient [CO_2] grown plants. Furthermore, elevated growth [CO_2] enhanced carbon and nitrogen uptake and increased foliage C:N ratio. These modifications were somewhat different among the clones, highlighting an important genetic source of variation in these patterns.
- Long-term growth [CO_2] modulated basal isoprene emission rate by altering DMADP supply and ISPS activity. Under the growth conditions of light intensity of 500 μmol m^{-2} s^{-1} and temperature of 30 °C, elevated growth [CO_2] did not significantly alter isoprene emission rate either measured at [CO_2] of 380 μmol mol^{-1} or 780 μmol mol^{-1}, but it decreased DMADP pool size and increased ISPS activity. This result implies that isoprene emission rate did not change because the decreased DMADP pool size was partly compensated by increased ISPS activity under moderate temperature and light conditions. Furthermore, when measurement [CO_2] from 380 μmol mol^{-1} changed into 780 μmol mol^{-1}, for both treatments, photosynthesis rate increased, isoprene emission rate and DMADP pool size decreased, while ISPS only depended on growth [CO_2]. These data indicated that DMADP pool size acclimated to growth [CO_2] and also was affected by instantaneous measurement [CO_2] under non-stress conditions.
- Elevated growth [CO_2] obviously enhanced hybrid aspen foliar photosynthetic and isoprene emission capacity. Elevated growth [CO_2] resulted in significantly higher maximum carboxylase activity of Rubisco (V_{cmax}) and capacity for photosynthetic electron transport (J_{max}), light-saturated net assimilation rate (A_{max}), initial quantum yield for CO_2 (Φ) and initial quantum yield for photosynthetic electron transport (Φ_J). Elevated [CO_2] grown plants had significantly higher maximum isoprene emission rate ($I_{max,ci}$) with corresponding lower intercellular CO_2 concentration for peak isoprene emission rate ($C_{i,max}$). Furthermore, elevated [CO_2] resulted in higher light-saturated isoprene emission rate ($I_{max,Q}$), while the quantum efficiency

was lower, especially at higher CO_2 measurement concentration, indicating reduced efficiency for use of photosynthetic electron transport in isoprene emission under moderate conditions.

- Elevated growth CO_2 resulted in higher stress resistance in hybrid aspen. During the heat stress, when temperature was increased from 30 °C to 50 °C, net assimilation rate was totally inhibited, while isoprene emission rate increased under both treatments. However, the plants grown under elevated [CO_2] had a lower reduction in net assimilation rate with greater enhancement of isoprene emissions, in particular, under the measurement [CO_2] of 780 μmol mol^{-1} and strong light intensity of 2000 μmol m^{-2} s^{-1}. There was a linear relationship between the reduction of net assimilation rate and the increase of isoprene emission rate, but the slope of this relationship was higher in ambient [CO_2] grown plants than in plants grown under elevated [CO_2], either measured at [CO_2] of 380 μmol mol^{-1} or 780 μmol mol^{-1}. This evidence collectively indicates that plants grown under elevated [CO_2] had higher heat stress resistance.
- Control on isoprene emission was shared between the environment temperature and foliage senescence during autumn. Photosynthetic rate, isoprene emission rate and DMADP pool size decreased rapidly accompanied with reduction of environmental temperature and foliage senescence. The decrease of photosynthetic rate and isoprene emission rate were mainly associated with leaf nitrogen content, but photosynthesis rate decreased faster than isoprene emission rate. The results demonstrated that the capacity for isoprene emissions can adjust to environmental conditions in senescing leaves as well, but the responsiveness is low compared with mid-season and is also affected by stress.
- The inhibition of isoprene emission by elevated [CO_2] disappeared under heat stress and high light, and isoprene emission rate was correlated with growth [CO_2]. We hypothesized that under stress when photosynthesis is reduced, (1) multiple carbon sources are mobilized for isoprene biosynthesis; and (2) isoprene biosynthesis has priority for DMADP over the biosynthesis of other pigments and higher isoprenoids in DXP pathway. Such or similar regulation is needed if isoprene functions as a protective gas involved in protection of photosystems. The plants grown under elevated [CO_2] had greater starch grains and higher soluble sugar concentration than plants

grown under ambient [CO_2], indirectly supporting the hypothesis that large carbon reserves might be necessary for isoprene formation under heat stress when photosynthesis is inhibited.

- Long-term elevated growth [CO_2] modulates plant growth and physiological process in multiple levels. Enhanced isoprene emission capacity and stress resistance may contribute to higher emission rate under stress, and such acclimation effects need to consideration in modelling isoprene emissions under future conditions.

Overall, we found that elevated growth [CO_2] affects isoprene emission at multiple scales, and that the acclimation effects importantly alter abiotic stress tolerance. We suggest that large-scale free air CO_2 enrichment (FACE) studies are needed to monitor long-term changes in canopy structure (leaf area index and biomass) to CO_2 enrichment and couple this to isoprene emission measurements.

Our experiments were conduction under moderate growth conditions and high nutrient supply. Given that nutrient supply influences the balance between "morphological upregulation" and "downregulation of photosynthesis", we also recommend to include different nutrient supply treatments in studies on elevated [CO_2] effects on isoprene emissions. Overall, we believe that these investigations will contribute to process-based understanding of isoprene emission and development of mechanistic models of isoprene emission.

REFERENCES

Affek HP, Yakir D (2002) Protection by isoprene against singlet oxygen in leaves. *Plant Physiology,* **129,** 269-277.

Affek HP, Yakir D (2003) Natural abundance carbon isotope composition of isoprene reflects incomplete coupling between isoprene synthesis and photosynthetic carbon flow. *Plant Physiology,* **131,** 1727-1736.

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. *New Phytologist,* **165,** 351-371.

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising CO_2 : mechanisms and environmental interactions. *Plant Cell and Environment,* **30,** 258-270.

Ainsworth EA, Rogers A, Blum H, Nosberger J, Long SP (2003) Variation in acclimation of photosynthesis in Trifolium repens after eight years of exposure to Free Air CO_2 Enrichment (FACE). *Journal of Experimental Botany,* **54,** 2769-2774.

Arneth A, Miller PA, Scholze M, Hickler T, Schurgers G, Smith B, Prentice IC (2007) CO_2 inhibition of global terrestrial isoprene emissions: Potential implications for atmospheric chemistry. *Geophysical Research Letters,* **34**, L18813.

Arneth A, Monson RK, Schurgers G, Niinemets U, Palmer PI (2008a) Why are estimates of global terrestrial isoprene emissions so similar (and why is this not so for monoterpenes)? *Atmospheric Chemistry and Physics,* **8,** 4605-4620.

Arneth A, Schurgers G, Hickler T, Miller PA (2008b) Effects of species composition, land surface cover, CO_2 concentration and climate on isoprene emissions from European forests. *Plant Biology,* **10,** 150-162.

Balogh G, Maulucci G, Gombos I, *et al.* (2011) Heat stress causes spatially-distinct membrane re-modelling in K562 leukemia cells. *PLoS ONE,* **6**, e21182.

Beers EP, McDowell JM (2001) Regulation and execution of programmed cell death in response to pathogens, stress and developmental cues. *Current Opinion in Plant Biology,* **4,** 561-567.

Behnke K, Ehlting B, Teuber M, *et al.* (2007) Transgenic, non-isoprene emitting poplars don't like it hot. *Plant Journal,* **51,** 485-499.

Behnke K, Loivamäki M, Zimmer I, Rennenberg H, Schnitzler J-P, Louis S (2010) Isoprene emission protects photosynthesis in sunfleck exposed grey poplar. *Photosynthesis Research,* **104,** 5-17.

Bertalanffy Lv (1957) Quantitative laws in metabolism and growth. *The Quarterly Review of Biology,* **32,** 217-231.

Bick JA, Lange BM (2003) Metabolic cross talk between cytosolic and plastidial pathways of isoprenoid biosynthesis: unidirectional transport of intermediates across the chloroplast envelope membrane. *Archives of Biochemistry and Biophysics,* **415,** 146-154.

Brilli F, Barta C, Fortunati A, Lerdau M, Loreto F, Centritto M (2007) Response of isoprene emission and carbon metabolism to drought in white poplar (Populus alba) saplings. *New Phytologist,* **175,** 244-254.

Brüggemann N, Schnitzler JP (2002) Comparison of isoprene emission, intercellular isoprene concentration and photosynthetic performance in water-limited oak (Quercus pubescens Willd. and Quercus robur L.) saplings. *Plant Biology,* **4,** 456-463.

Calfapietra C, Ainsworth EA, Beier C, *et al.* (2010) Challenges in elevated CO_2 experiments on forests. *Trends in Plant Science,* **15,** 5-10.

Calfapietra C, Scarascia Mugnozza G, Karnosky DF, Loreto F, Sharkey TD (2008) Isoprene emission rates under elevated CO_2 and O_3 in two field-grown aspen clones differing in their sensitivity to O_3. *New Phytologist,* **179,** 55-61.

Calfapietra C, Tulva I, Eensalu E, Perez M, De Angelis P, Scarascia-Mugnozza G, Kull O (2005) Canopy profiles of photosynthetic parameters under elevated CO_2 and N fertilization in a poplar plantation. *Environmental Pollution,* **137,** 525-535.

Centritto M, Brilli F, Fodale R, Loreto F (2011) Different sensitivity of isoprene emission, respiration and photosynthesis to high growth temperature coupled with drought stress in black poplar (Populus nigra) saplings. *Tree Physiology,* **31,** 275-286.

Centritto M, Jarvis PG (1999) Long-term effects of elevated carbon dioxide concentration and provenance on four clones of Sitka spruce (Picea sitchensis). II. Photosynthetic capacity and nitrogen use efficiency. *Tree Physiology,* **19,** 807-814.

Centritto M, Nascetti P, Petrilli L, Raschi A, Loreto F (2004) Profiles of isoprene emission and photosynthetic parameters in hybrid poplars exposed to free-air CO_2 enrichment. *Plant, Cell & Environment,* **27,** 403-412.

Chen GY, Yong ZH, Liao Y, *et al.* (2005) Photosynthetic acclimation in rice leaves to free-air CO_2 enrichment related to both ribulose-1,5-bisphosphate carboxylation limitation and ribulose-1,5-bisphosphate regeneration limitation. *Plant and Cell Physiology,* **46,** 1036-1045.

Cinege G, Louis S, Haensch R, Schnitzler J-P (2009) Regulation of isoprene synthase promoter by environmental and internal factors. *Plant Molecular Biology,* **69,** 593-604.

Copolovici L, Niinemets U (2010) Flooding induced emissions of volatile signalling compounds in three tree species with differing waterlogging tolerance. Plant Cell and Environment, **33**, 1582-1594.

Darbah JNT, Sharkey TD, Calfapietra C, Karnosky DF (2010) Differential response of aspen and birch trees to heat stress under elevated carbon dioxide. *Environmental Pollution,* **158,** 1008-1014.

de Graaff M-A, van Groenigen K-J, Six J, Hungate B, van Kessel C (2006) Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. *Global Change Biology,* **12,** 2077-2091.

Delwiche CF, Sharkey TD (1993) Rapid appearance of ^{13}C in biogenic isoprene when $^{13}CO_2$ is fed to intact leaves. *Plant, Cell & Environment,* **16,** 587-591.

Ekberg A, Arneth A, Hakola H, Hayward S, Holst T (2009) Isoprene emission from wetland sedges. *Biogeosciences,* **6,** 601-613.

Fang CW, Monson RK, Cowling EB (1996) Isoprene emission, photosynthesis, and growth in sweetgum (Liquidambar styraciflua) seedlings exposed to short- and long-term drying cycles. *Tree Physiology,* **16,** 441-446.

Farquhar GD, Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. *Planta,* **149,** 78-90.

Fisher AJ, Rosenstiel TN, Shirk MC, Fall R (2001) Nonradioactive assay for cellular dimethylallyl diphosphate. *Analytical Biochemistry,* **292,** 272-279.

Fortunati A, Barta C, Brilli F, Centritto M, Zimmer I, Schnitzler J-P, Loreto F (2008) Isoprene emission is not temperature-dependent during and after severe drought-stress: A physiological and biochemical analysis. *Plant Journal,* **55,** 687-697.

Funk JL, Mak JE, Lerdau MT (2004) Stress-induced changes in carbon sources for isoprene production in *Populus deltoides*. *Plant, Cell & Environment,* **27,** 747-755.

Goldstein AH, Galbally IE (2007) Known and unexplored organic constituents in the earth's atmosphere. *Environmental Science & Technology,* **41,** 1514-1521.

Guenther A, Hewitt CN, Erickson D*, et al.* (1995) A global model of natural volatile organic compound emissions. *J. Geophys. Res.,* **100,** 8873-8892.

Guenther A, Karl T, Harley P, Wiedinmyer C, Palmer PI, Geron C (2006) Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature). *Atmos. Chem. Phys.,* **6,** 3181-3210.

Guenther AB, Zimmerman PR, Harley PC, Monson RK, Fall R (1993) Isoprene and monoterpene emission rate variability: Model evaluations and sensitivity analyses. *Journal of Geophysical Research: Atmospheres,* **98,** 12609-12617.

Haikio E, Freiwald V, Julkunen-Tiitto R, Beuker E, Holopainen T, Oksanen E (2009) Differences in leaf characteristics between ozone-sensitive and ozone-tolerant hybrid aspen (Populus tremula x Populus tremuloides) clones. *Tree Physiology,* **29,** 53-66.

Hald S, Pribil M, Leister D, Gallois P, Johnson GN (2008) Competition between linear and cyclic electron flow in plants deficient in Photosystem I. *Biochimica et Biophysica Acta-Bioenergetics,* **1777,** 1173-1183.

Harley P, Guenther A, Zimmerman P (1996) Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (Liquidambar styraciflua) leaves. *Tree Physiology,* **16,** 25-32.

Harley PC, Monson RK, Lerdau MT (1999) Ecological and evolutionary aspects of isoprene emission from plants. *Oecologia,* **118,** 109-123.

Heald CL, Wilkinson MJ, Monson RK, Alo CA, Wang G, Guenther A (2009) Response of isoprene emission to ambient CO_2 changes and implications for global budgets. *Global Change Biology,* **15,** 1127-1140.

Henze DK, Seinfeld JH (2006) Global secondary organic aerosol from isoprene oxidation. *Geophysical Research Letters,* **33**: L09812.

Hopkins M, Taylor C, Liu Z, Ma F, McNamara L, Wang T-W, Thompson JE (2007) Regulation and execution of molecular disassembly and catabolism during senescence. *New Phytologist,* **175,** 201-214.

Huang J-G, Bergeron Y, Denneler B, Berninger F, Tardif J (2007) Response of forest trees to increased atmospheric CO_2. *Critical Reviews in Plant Sciences,* **26,** 265-283.

Jablonski LM, Wang XZ, Curtis PS (2002) Plant reproduction under elevated CO_2 conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytologist,* **156,** 9-26.

Johnson DW (2006) Progressive N limitation in forests: Review and implications for long-term responses to elevated CO_2. *Ecology,* **87,** 64-75.

Kanakidou M, Seinfeld JH, Pandis SN, *et al.* (2005) Organic aerosol and global climate modelling: a review. *Atmospheric Chemistry and Physics,* **5,** 1053-1123.

Karl T, Fall R, Rosenstiel TN, Prazeller P, Larsen B, Seufert G, Lindinger W (2002) On-line analysis of the (CO_2)-C-13 labelling of leaf isoprene suggests multiple subcellular origins of isoprene precursors. *Planta,* **215,** 894-905.

Keskitalo J, Bergquist G, Gardestrom P, Jansson S (2005) A cellular timetable of autumn senescence. *Plant Physiology,* **139,** 1635-1648.

Kiendler-Scharr A, Zhang Q, Hohaus T, *et al.* (2009) Aerosol mass spectrometric features of biogenic SOA: observations from a plant chamber and in rural atmospheric environments. *Environmental Science & Technology,* **43,** 8166-8172.

Kratsch HA, Wise RR (2000) The ultrastructure of chilling stress. *Plant Cell and Environment,* **23,** 337-350.

Kroll JH, Ng NL, Murphy SM, Flagan RC, Seinfeld JH (2006) Secondary organic aerosol formation from isoprene photooxidation. *Environmental Science & Technology,* **40,** 1869-1877.

Kuhn U, Rottenberger S, Biesenthal T, *et al.* (2004) Seasonal differences in isoprene and light-dependent monoterpene emission by Amazonian tree species. *Global Change Biology,* **10,** 663-682.

Kulmala M, Vehkamaki H, Petaja T, *et al.* (2004) Formation and growth rates of ultrafine atmospheric particles: a review of observations. *Journal of Aerosol Science,* **35,** 143-176.

Kuzma J, Fall R (1993) Leaf Isoprene emission rate is dependent on leaf development and the level of isoprene synthase. *Plant Physiology,* **101,** 435-440.

Kwan AJ, Chan AWH, Ng NL, Kjaergaard HG, Seinfeld JH, Wennberg PO (2012) Peroxy radical chemistry and OH radical production during the NO_3-initiated oxidation of isoprene. *Atmospheric Chemistry and Physics,* **12,** 7499-7515.

Laothawornkitkul J, Paul ND, Vickers CE, Possell M, Taylor JE, Mullineaux PM, Hewitt CN (2008) Isoprene emissions influence herbivore feeding decisions. *Plant Cell and Environment,* **31,** 1410-1415.

Laule O, Furholz A, Chang HS*, et al.* (2003) Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences of the United States of America,* **100,** 6866-6871.

Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR (2009) Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *Journal of Experimental Botany,* **60,** 2859-2876.

Lehning A, Zimmer I, Steinbrecher R, BrÜGgemann N, Schnitzler JP (1999) Isoprene synthase activity and its relation to isoprene emission in Quercus robur L. leaves. *Plant, Cell & Environment,* **22,** 495-504.

Lelieveld J, Butler TM, Crowley JN*, et al.* (2008) Atmospheric oxidation capacity sustained by a tropical forest. *Nature,* **452,** 737-740.

Li Z, Ratliff EA, Sharkey TD (2011) Effect of temperature on postillumination isoprene emission in oak and poplar. *Plant Physiology,* **155,** 1037-1046.

Liberloo M, Tulva I, Raim O, Kull O, Ceulemans R (2007) Photosynthetic stimulation under long-term CO_2 enrichment and fertilization is sustained across a closed Populus canopy profile (EUROFACE). *New Phytologist,* **173,** 537-549.

Lichtenthaler HK (1999) The 1-deoxy-d-xylulose-5-phosphate pathway of isoprenoid biosyhtesis in plants. *Annual Review of Plant Physiology and Plant Molecular Biology,* **50,** 47-65.

Lichtenthaler HK (2007) Biosynthesis, accumulation and emission of carotenoids, alpha-tocopherol, plastoquinone, and isoprene in leaves under high photosynthetic irradiance. *Photosynthesis Research,* **92,** 163-179.

Lichtenthaler HK, Schwender Jr, Disch A, Rohmer M (1997) Biosynthesis of isoprenoids in higher plant chloroplasts proceeds via a mevalonate-independent pathway. *FEBS Letters,* **400,** 271-274.

Lin JS, Wang GX (2002) Doubled CO_2 could improve the drought tolerance better in sensitive cultivars than in tolerant cultivars in spring wheat. *Plant Science,* **163,** 627-637.

Litvak ME, Loreto F, Harley PC, Sharkey TD, Monson RK (1996) The response of isoprene emission rate and photosynthetic rate to photon flux and nitrogen supply in aspen and white oak trees. *Plant, Cell & Environment,* **19,** 549-559.

Liu ZG, Li FR (2003) The generalized Chapman-Richards function and applications to tree and stand growth. *Journal of Forestry Research,* **14,** 19-26.

Loivamaeki M, Louis S, Cinege G, Zimmer I, Fischbach RJ, Schnitzler J-P (2007) Circadian rhythms of isoprene biosynthesis in Grey poplar leaves. *Plant Physiology,* **143,** 540-551.

Loivamaki M, Mumm R, Dicke M, Schnitzler JP (2008) Isoprene interferes with the attraction of bodyguards by herbaceous plants. *Proceedings of the National Academy of Sciences of the United States of America,* **105,** 17430-17435.

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants FACE the future. *Annual review of plant biology,* **55,** 591-628.

Loreto F, Centritto M, Barta C, Calfapietra C, Fares S, Monson RK (2007) The relationship between isoprene emission rate and dark respiration rate in white poplar (*Populus alba* L.) leaves. *Plant, Cell & Environment,* **30,** 662-669.

Loreto F, Delfine S (2000) Emission of isoprene from salt-stressed Eucalyptus globulus leaves. *Plant Physiology,* **123,** 1605-1610.

Loreto F, Mannozzi M, Maris C, Nascetti P, Ferranti F, Pasqualini S (2001a) Ozone quenching properties of isoprene and its antioxidant role in leaves. *Plant Physiology,* **126,** 993-1000.

Loreto F, Schnitzler J-P (2010) Abiotic stresses and induced BVOCs. *Trends in plant science,* **15,** 154-166.

Loreto F, Sharkey T (1993) On the relationship between isoprene emission and photosynthetic metabolites under different environmental conditions. *Planta,* **189,** 420-424.

Loreto F, Velikova V (2001b) Isoprene Produced by Leaves Protects the Photosynthetic Apparatus against Ozone Damage, Quenches Ozone Products, and Reduces Lipid Peroxidation of Cellular Membranes. *Plant Physiology,* **127,** 1781-1787.

Luo YQ, Hui DF, Zhang DQ (2006) Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems: A meta-analysis. *Ecology,* **87,** 53-63.

Luo YQ, Sims DA, Griffin KL (1998) Nonlinearity of photosynthetic responses to growth in rising atmospheric CO_2: an experimental and modelling study. *Global Change Biology,* **4,** 173-183.

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: An overview. *Archives of Biochemistry and Biophysics,* **444,** 139-158.

Mayrhofer S, Teuber M, Zimmer I, Louis S, Fischbach RJ, Schnitzler RP (2005) Diurnal and seasonal variation of isoprene biosynthesis-related genes in Grey poplar leaves. *Plant Physiology,* **139,** 474-484.

Miyazawa S-I, Warren CR, Turpin DH, Livingston NJ (2011) Determination of the site of CO_2 sensing in poplar: is the area-based N content and anatomy of new leaves determined by their immediate CO_2 environment or by the CO_2 environment of mature leaves? *Journal of Experimental Botany,* **62,** 2787-2796.

Monson R K., Wilkinson MJ., Monson ND., Trahan N., Lee S., Rosenstiel TR., R. F (2009) Biochemical control on the CO_2 response of leaf isoprene emisson: an alternative view of Sanadze's double carboxylation scheme. *Aanalysis of Agarian Science,* **7**, 21-29.

Monson RK, Fall R (1989) Isoprene emission from aspen leaves : influence of environment and relation to photosynthesis and photorespiration. *Plant Physiology,* **90,** 267-274.

Monson RK, Harley PC, Litvak ME, Wildermuth M, Guenther AB, Zimmerman PR, Fall R (1994) Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves. *Oecologia,* **99,** 260-270.

Monson RK, Jones RT, Rosenstiel TN, Schnitzler J-P (2013) Why only some plants emit isoprene. *Plant, Cell & Environment***, 36**, 503-516.

Monson RK, Trahan N, Rosenstiel TN*, et al.* (2007) Isoprene emission from terrestrial ecosystems in response to global change: minding the gap between models and observations. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences,* **365,** 1677-1695.

Niinemets Ü (2010a) A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. *Ecological Research,* **25,** 693-714.

Niinemets Ü (2010b) Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: Past stress history, stress interactions, tolerance and acclimation. Forest Ecology and Management, **260**, 1623-1639.

Niinemets U, Arneth A, Kuhn U, Monson RK, Peñuelas J, Staudt M (2010c) The emission factor of volatile isoprenoids: stress, acclimation, and developmental responses. *Biogeosciences,* **7,** 2203-2223.

Niinemets Ü, Copolovici L, Hüve K (2010d) High within-canopy variation in isoprene emission potentials in temperate trees: Implications for predicting canopy-scale isoprene fluxes. *J. Geophys. Res.,* **115,** G04029.

Niinemets U, Monson RK, Arneth A*, et al.* (2010e) The leaf-level emission factor of volatile isoprenoids: caveats, model algorithms, response shapes and scaling. *Biogeosciences,* **7,** 1809-1832.

Niinemets Ü, Tenhunen JD, Harley PC, Steinbrecher R (1999) A model of isoprene emission based on energetic requirements for isoprene synthesis and leaf photosynthetic properties for *Liquidambar* and *Quercus*. *Plant, Cell & Environment,* **22,** 1319-1335.

Norby RJ, Luo YQ (2004) Evaluating ecosystem responses to rising atmospheric CO_2 and global warming in a multi-factor world. *New Phytologist,* **162,** 281-293.

Nowak RS, Ellsworth DS, Smith SD (2004) Functional responses of plants to elevated atmospheric CO_2 - do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist,* **162,** 253-280.

Ögren E, Evans JR (1993) Photosynthetic light-response curves. 1. The influence of CO_2 partial pressure and leaf inversion. *Planta,* **189,** 182-190.

Oksanen E, Sober J, Karnosky DF (2001) Impacts of elevated CO_2 and/or O_3 on leaf ultrastructure of aspen (Populus tremuloides) and birch (Betula papyrifera) in the Aspen FACE experiment. *Environmental Pollution,* **115,** 437-446.

Pacifico F, Harrison SP, Jones CD, Sitch S (2009) Isoprene emissions and climate. *Atmospheric Environment,* **43,** 6121-6135.

Pasqua G, Monacelli B, Manfredini C, Loreto FPerez G. (2002) The Role of Isoprenoid Accumulation and Oxidation in Sealing Wounded Needles of Mediterranean Pines. *Plant Science* **163,** 355-359.

Paulot F, Crounse JD, Kjaergaard HG, Kuerten A, St Clair JM, Seinfeld JH, Wennberg PO (2009) Unexpected epoxide formation in the gas-phase photooxidation of isoprene. *Science,* **325,** 730-733.

Pegoraro E, Rey A, Bobich EG, Barron-Gafford G, Grieve KA, Malhi Y, Murthy R (2004) Effect of elevated CO_2 concentration and vapour pressure deficit on isoprene emission from leaves of Populus deltoides during drought. *Functional Plant Biology,* **31,** 1137-1147.

Peñuelas J, Llusia J, Asensio D, Munne-Bosch S (2005) Linking isoprene with plant thermotolerance, antioxidants and monoterpene emissions. *Plant Cell and Environment,* **28,** 278-286.

Pienaar LV, Turnbull KJ (1973) The Chapman-Richards generalization of Von Bertalanffy's growth model for basal area growth and yield in even - aged stands. *Forest Science,* **19,** 2-22.

Possell M, Hewitt CN (2009) Gas exchange and photosynthetic performance of the tropical tree Acacia nigrescens when grown in different CO_2 concentrations. Planta, **229**, 837-846.

Possell M, Heath J, Nicholas Hewitt C, Ayres E, Kerstiens G (2004) Interactive effects of elevated CO_2 and soil fertility on isoprene emissions from Quercus robur. *Global Change Biology,* **10,** 1835-1843.

Possell M, Hewitt CN (2011) Isoprene emissions from plants are mediated by atmospheric CO_2 concentrations. *Global Change Biology,* **17,** 1595-1610.

Possell M, Nicholas Hewitt C, Beerling DJ (2005) The effects of glacial atmospheric CO_2 concentrations and climate on isoprene emissions by vascular plants. *Global Change Biology,* **11,** 60-69.

Rasulov B, Copolovici L, Laisk A, Niinemets Ü (2009a) Postillumination isoprene emission: in vivo measurements of dimethylallyldiphosphate pool size and isoprene synthase kinetics in aspen leaves. *Plant Physiology,* **149,** 1609-1618.

Rasulov B, Hüve K, Bichele I, Laisk A, Niinemets Ü (2010) Temperature response of isoprene emission in vivo reflects a combined effect of substrate limitations and isoprene synthase activity: a kinetic analysis. *Plant Physiology,* **154,** 1558-1570.

Rasulov B, Hueve K, Vaelbe M, Laisk A, Niinemets U (2009b) Evidence that light, carbon dioxide, and oxygen dependencies of leaf isoprene emission are driven by energy status in hybrid aspen. *Plant Physiology,* **151,** 448-460.

Rogers A, Ainsworth EA, Leakey ADB (2009) Will elevated carbon dioxide concentration amplify the benefits of nitrogen ffixation in legumes? *Plant Physiology,* **151,** 1009-1016.

Rosenstiel TN, Potosnak MJ, Griffin KL, Fall R, Monson RK (2003) Increased CO_2 uncouples growth from isoprene emission in an agriforest ecosystem. *Nature,* **421,** 256-259.

Sanadze (2004) Biogenic Isoprene (A Review). *Russian Journal of Plant Physiology,* **51**, 729-741.

Sanadze G (2010) Photobiosynthesis of isoprene as an example of leaf excretory function in the light of contemporary thermodynamics. *Russ J Plant Physiol,* **57,** 1-6.

Sanderson MG, Jones CD, Collins WJ, Johnson CE, Derwent RG (2003) Effect of climate change on isoprene emissions and surface ozone levels. *Geophysical Research Letters,* **30**, 1936.

Sanz-Saez A, Erice G, Aranjuelo I, Nogues S, Irigoyen JJ, Sanchez-Diaz M (2010) Photosynthetic down-regulation under elevated CO_2 exposure can be prevented by nitrogen supply in nodulated alfalfa. *Journal of Plant Physiology,* **167,** 1558-1565.

Sasaki K, Ohara K, Yazaki K (2005) Gene expression and characterization of isoprene synthase from Populus alba. *FEBS Letters,* **579,** 2514-2518.

Sasaki K, Saito T, Laemsae M, *et al.* (2007) Plants utilize isoprene emission as a thermotolerance mechanism. *Plant and Cell Physiology,* **48,** 1254-1262.

Schnitzler JP, Graus M, Kreuzwieser J, Heizmann U, Rennenberg H, Wisthaler A, Hansel A (2004) Contribution of different carbon sources to isoprene biosynthesis in poplar leaves. *Plant Physiology,* **135,** 152-160.

Scholefield PA, Doick KJ, Herbert BMJ, Hewitt CN, Schnitzler JP, Pinelli P, Loreto F (2004) Impact of rising CO_2 on emissions of volatile organic compounds: isoprene emission from Phragmites australis growing at elevated CO_2 in a natural carbon dioxide spring. *Plant Cell and Environment,* **27,** 393-401.

Schwanz P, Picon C, Vivin P, Dreyer E, Guehl JM, Polle A (1996) Responses of antioxidative systems to drought stress in pendunculate oak and maritime pine as modulated by elevated CO_2. *Plant Physiology,* **110,** 393-402.

Schwender J, Gemunden C, Lichtenthaler HK (2001) Chlorophyta exclusively use the 1-deoxyxylulose 5-phosphate/2-C-methylerythritol 4-phosphate pathway for the biosynthesis of isoprenoids. *Planta,* **212,** 416-423.

Sharkey T, Loreto F (1993) Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. *Oecologia,* **95,** 328-333.

Sharkey TD, Chen XY, Yeh S (2001a) Isoprene increases thermotolerance of fosmidomycin-fed leaves. *Plant Physiology,* **125,** 2001-2006.

Sharkey TD, Loreto F, Delwiche CF (1991) High carbon dioxide and sun/shade effects on isoprene emission from oak and aspen tree leaves. *Plant, Cell & Environment,* **14,** 333-338.

Sharkey TD, Singsaas EL, Vanderveer PJ, Geron C (1996) Field measurements of isoprene emission from trees in response to temperature and light. *Tree Physiology,* **16,** 649-654.

Sharkey TD, Wiberley AE, Donohue AR (2008) Isoprene emission from plants: Why and how. *Annals of Botany,* **101,** 5-18.

Sharkey TD, Yeh S (2001b) Isoprene emission frm plants. *Annual Review of Plant Physiology and Plant Molecular Biology,* **52,** 407-436.

Sims DA, Seemann JR, Luo YQ (1998) Elevated CO_2 concentration has independent effects on expansion rates and thickness of soybean leaves across light and nitrogen gradients. *Journal of Experimental Botany,* **49,** 583-591.

Singsaas EL, Laporte MM, Shi JZ*, et al.* (1999) Kinetics of leaf temperature fluctuation affect isoprene emission from red oak (Quercus rubra) leaves. *Tree Physiology,* **19,** 917-924.

Singsaas EL, Lerdau M, Winter K, Sharkey TD (1997) Isoprene increases thermotolerance of isoprene-emitting species. *Plant Physiology,* **115,** 1413-1420.

Taraborrelli D, Lawrence MG, Butler TM, Sander R, Lelieveld J (2009) Mainz Isoprene Mechanism 2 (MIM2): An isoprene oxidation mechanism for regional and global atmospheric modelling. *Atmospheric Chemistry and Physics,* **9,** 2751-2777.

Taub DR, Seemann JR, Coleman JS (2000) Growth in elevated CO_2 protects photosynthesis against high-temperature damage. *Plant Cell and Environment,* **23,** 649-656.

Taub DR, Wang X (2008) Why are nitrogen concentrations in plant tissues lower under elevated CO_2? A critical examination of the hypotheses. *Journal of Integrative Plant Biology,* **50,** 1365-1374.

Terry GM, Stokes NJ, Hewitt CN, Mansfield TA (1995) Exposure to isoprene promotes flowering in plants. *Journal of Experimental Botany,* **46,** 1629-1631.

Teuber M, Zimmer I, Kreuzwieser J, Ache P, Polle A, Rennenberg H, Schnitzler JP (2008) VOC emissions of Grey poplar leaves as affected by salt stress and different N sources. *Plant Biology,* **10,** 86-96.

Thornley JHM (1976) Mathematical models in plant physiology. A quantitative approach to problems in plant and crop physiology. *Academic Press, London - New York - San Francisco*, XIV, 318 pp

Tognetti R, Johnson JD, Michelozzi M, Raschi A (1998) Response of foliar metabolism in mature trees of Quercus pubescens and Quercus ilex to long-term elevated CO_2. *Environmental and Experimental Botany,* **39,** 233-245.

Tosens T, Niinemets U, Vislap V, Eichelmann H, Castro Diez P (2012) Developmental changes in mesophyll diffusion conductance and photosynthetic capacity under different light and water availabilities in Populus tremula: how structure constrains function. *Plant Cell and Environment,* **35,** 839-856.

Trowbridge AM, Asensio D, Eller ASD*, et al.* (2012) Contribution of various carbon sources toward isoprene biosynthesis in poplar leaves mediated by altered atmospheric CO_2 concentrations. *PLoS ONE,* **7,** e32387.

Tuba Z, Lichtenthaler HK (2007) Long-term acclimation of plants to elevated CO_2 and its interaction with stresses. In *Stress Responses in Biology and Medicine: Stress of Life in Molecules, Cells, Organisms, and Psychosocial Communities* Vol. 1113 (eds Csermely P, Korcsmaros T, Sulyok K), pp. 135-146.

Velikova V, Edreva A, Loreto F (2004) Endogenous isoprene protects Phragmites australis leaves against singlet oxygen. *Physiologia Plantarum,* **122,** 219-225.

Velikova V, Loreto F (2005) On the relationship between isoprene emission and thermotolerance in *Phragmites australis* leaves exposed to high temperatures and during the recovery from a heat stress. *Plant, Cell & Environment,* **28,** 318-327.

Velikova V, Tsonev T, Barta C*, et al.* (2009) BVOC emissions, photosynthetic characteristics and changes in chloroplast ultrastructure of Platanus orientalis L. exposed to elevated CO_2 and high temperature. *Environmental Pollution,* **157,** 2629-2637.

Velikova V, Varkonyi Z, Szabo M*, et al.* (2011) Increased thermostability of thylakoid membranes in isoprene-emitting leaves probed with three biophysical techniques. *Plant Physiology,* **157,** 905-916.

Vickers C, Possell M, Nicholas Hewitt C, Mullineaux P (2010) Genetic structure and regulation of isoprene synthase in Poplar (*Populus* spp.). *Plant Molecular Biology,* **73,** 547-558.

Vickers CE, Gershenzon J, Lerdau MT, Loreto F (2009) A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nature Chemical Biology,* **5,** 283-291.

Walther GR, Post E, Convey P*, et al.* (2002) Ecological responses to recent climate change. *Nature,* **416,** 389-395.

Wang C, Ma XL, Hui Z, Wang W (2008a) Glycine betaine improves thylakoid membrane function of tobacco leaves under low-temperature stress. *Photosynthetica,* **46,** 400-409.

Wang G, Chen K, Chen L, Hu C, Zhang D, Liu Y (2008b) The involvement of the antioxidant system in protection of desert cyanobacterium Nostoc sp against UV-B radiation and the effects of exogenous antioxidants. *Ecotoxicology and Environmental Safety,* **69,** 150-157.

Wang X, Taub DR (2010) Interactive effects of elevated carbon dioxide and environmental stresses on root mass fraction in plants: A meta-analytical synthesis using pairwise techniques. *Oecologia,* **163,** 1-11.

Wiberley AE, Donohue AR, Westphal MM, Sharkey TD (2009) Regulation of isoprene emission from poplar leaves throughout a day. *Plant Cell and Environment,* **32,** 939-947.

Wiedinmyer C, Tie X, Guenther A, Neilson R, Granier C (2006) Future changes in biogenic isoprene emissions: How might they affect regional and global atmospheric chemistry? *Earth Interactions,* **10**, 1-19.

Wilkinson MJ, Monson RK, Trahan N, *et al.* (2009) Leaf isoprene emission rate as a function of atmospheric CO_2 concentration. *Global Change Biology,* **15,** 1189-1200.

Willmer PG, Nuttman CV, Raine NE, *et al.* (2009) Floral volatiles controlling ant behaviour. *Functional Ecology,* **23,** 888-900.

Yang RC, Kozak A, Smith JHG (1978) The potential of Weibull-type functions as flexible growth curves. *Canadian Journal of Forest Research,* **8,** 424-431.

Yoshioka H, Bouteau F, Kawano T (2008) Discovery of oxidative burst in the field of plant immunity: Looking back at the early pioneering works and towards the future development. *Plant signaling & behavior,* **3,** 153-155.

Young PJ, Arneth A, Schurgers G, Zeng G, Pyle JA (2009) The CO_2 inhibition of terrestrial isoprene emission significantly affects future ozone projections. *Atmospheric Chemistry and Physics,* **9,** 2793-2803.

Yu QB, Pulkkinen P (2003) Genotype-environment interaction and stability in growth of aspen hybrid clones. *Forest Ecology and Management,* **173,** 25-35.

Zhang DY, Chen GY, Chen J, Yong ZH, Zhu JG, Xu DQ (2009) Photosynthetic acclimation to CO_2 enrichment related to ribulose-1,5-bisphosphate carboxylation limitation in wheat. *Photosynthetica,* **47,** 152-154.

SUMMARY IN ESTONIAN

KESKKONNATEGURITE MÕJU HAAVA (*Populus sp.*) ISOPREENI EMISSIOONILE

Sissejuhatus

Isopreen on üks enam levinud biogeenne orgaaniline lenduvühend (*BVOC – biogenic volatile organic compound*), mida eritavad paljud taimeliigid. Isopreen moodustab 40% nimetatud ühendite globaalsest kogusest, so 440-660 megatonni süsinikku aastas (Guenther *et al.*, 2006). Atmosfääris reageerib kõrge reaktiivsusega isopreen kiiresti hüdroksüülradikaalidega (OH), lämmastikoksiididega (NO_2) ja osooniga (Kanakidou *et al.*, 2005, Pacifico *et al.*, 2009). See mõjutab tugevasti osooni ja sekundaarse orgaanilise aerosooli (SOA) teket ja kontrollib osaliselt ka kasvuhoonegaasi, metaani, eluiga troposfääris. Seega on isopreen globaalsete protsesside potentsiaalne mõjutaja (1992; Claeys *et al.*, 2004; Hallquist *et al.*, 2009).

Varasemad uuringud on välja selgitanud, et isopreen täidab tähtsat bioloogilist rolli kaitstes taimi biootilise ja abiootilise stressi eest. On leitud, et isopreen kaitseb taime fotosünteesiaparaati mõõduka kuumastressi eest taimele langevat liigset energiat hajutades. Vastuseks tugevale kiirgusele ja kuumastressile aitab isopreen stabiliseerida biomembraani (Behnke *et al.*, 2010, Sharkey *et al.*, 2001). Isopreen toimib ka stressi tekitatud ja herbivooride tõrjumiseks mõeldud reaktiivse hapniku ühendite (*reactive oxygen species*) kahjutuks tegijana (Behnke *et al.*, 2010, Sharkey *et al.*, 2001). Vaatamata edusammudele on isopreeni kaitsemehhanismi toimimine siiani tervikuna siiski veel ebaselge (Affek *et al.*, 2002, Vickers *et al.*, 2009).

Isopreen tekib kloroplastides vastava ensüümi, isopreeni süntaasi (ISPS) katalüüsitud keemilise reaktsiooni käigus, kus dimetüülallüüldifosfaat (DMADP) muudetakse isopreeniks ning difosfaadiks. DMADP omakorda tekib 1-deoksü-d-ksüluloos-5-fosfaadist (DOXP) (Affek *et al.*, 2003, Lichtenthaler, 1999, Schwender *et al.*, 2001). Isopreeni teke on tihedasti seotud fotosünteesi käigus toodetud süsiniku metabolismiga.

Seega määrab isopreeni eritamist taimede poolt nii substraadi (DMADP) olemasolu kui ka ISPS-i aktiivsus. Eelnevad uuringud on välja selgitanud, et glütseeraldehüüd-3-fosfaadi (GA3P) algne substraat DOXP rajal biosünteesitud DMADP jaoks pärineb stressivabades lehtedes vastseotud CO_2-st (Affek *et al.*, 2003, Delwiche *et al.*, 1993, Trowbridge *et al.*, 2012). Hiljutine eksperiment märgistatud ^{13}C-ga kinnitas, et stressi tõttu limiteeritud fotosünteesi käigus kasutatakse isopreeni sünteesiks mitut erinevat süsinikuallikat, milleks on tärklise lagundamine kloroplastides, ksüleemis transporditavad süsivesikud ning tsütoplasma püruvaatide/ fosfoenoolpüruvaatide ekvivalendid (Brilli *et al.*, 2007, Funk *et al.*, 2004, Trowbridge *et al.*, 2012).

Taimede isopreeni sünteesi mõjutavad nii keskkonna võtmetegurid, valgus ja temperatuur, kui ka aastaaegade vaheldumisega seotud muutused lehestikus (lehtede vananemine) (Kuhn *et al.*, 2004; Loreto *et al.*, 1993, Trowbridge *et al.*, 2012). Noored või arenevad taimelehed eritavad isopreeni aeglaselt, sest nendes läheb DMADP peamiselt polüterpeenide ja pigmentide moodustamiseks, mida kasutatakse kõikides organismi membraanisüsteemides (Mayrhofer *et al.*, 2005). Samas on isopreeni eritamine täiskasvanud lehtedes kasvuaegse temperatuuri ja valguse kiirguse muutumisel reguleeritud ISPSi aktiivsusega ja DOXP raja kaudu moodustusnud DMADP-ga. Isopreeni eritamine hakkab langema lehtede täiskasvanuks saamisel ja kestab lehtede vananedes. Seda on täheldatud mitmete erinevate taimeliikide puhul. Vananemise käigus väheneb lehe füsioloogiline aktiivsus, kuid on ebaselge, kas sellel etapil isopreeni eritamise võime kohaneb keskkonnatingimuste peioodiliste muutustega või väheneb ühtlaselt (Vickers *et al.*, 2009, Niinemets *et al.*, 2010b, Loreto *et al.*, 2010).

CO_2 suurenenud sisalduse mõju isopreeni eritamise kiirusele on kirjeldatud alates 1960ndatest aastatest (Sharkey *et al.*, 2001, Wilkinson *et al.*, 2009). Tööstusrevolutsiooni eelse ajaga võrreldes on CO_2 sisaldus (edaspidi [CO_2]) atmosfääris tõusnud 280st miljondikosast (*ppm*) kuni 385 miljondikosani tänapäeval ja ennustatavalt kasvab veelgi. [CO_2] kasv atmosfääris on oluline tegur, mis võib mõjutada isopreeni eritamist suures ulatuses (de Graaff *et al.*, 2006, Long *et al.*, 2004, Luo *et al.*, 2006). Eelnevad uuringud on välja selgitanud, et lühi- ja pikaajaline [CO_2] muutus mõjutab isopreeni eritamise kiirust erinevalt. Lühiajaliselt suurenev [CO_2] pärsib isopreeni eritamist, kuid mudelid,

mille koostamisel on arvestatud tuleviku kõrgemate atmosfäärse CO_2 sisaldustega, ennustavad isopreeni eritamise vähenemist. Pikaajalise [CO_2] kasvu mõju isopreeni eritamisele on uuritud erinevatel taimedel mitmesugustes eksperimentaalsetes tingimustes. Enamike uuringute tulemusel on suurenenud [CO_2] puhul jälgitav isopreemi eritamise kiiruse vähenemine. On ka uuringuid, mis näitavad, et suurenenud [CO_2] mõju isopreeni eritamisele on kas mõõdukas või isegi puudub, või on hoopis isopreeni eritamise võimet suurendav. Tänapäeval on [CO_2] atmosfääris kaugelt allpool fotosünteesi küllastuspiiri. Seega on atmosfääris CO_2 tõusuga oodata fotosünteesi intensiivistumist, süsiniku sidumist ja taimede kasvu. On ka ennustatud, et [CO_2] kasvuga kaasneb põud ja suvised kõrged temperatuurid, mis võivad mõjutada taimede kohastumist ja vastupidavust stressile. Seega on atmosfääris [CO_2] tõusu mõju taimedele mitmesugune, mis muudab isopreeni eritamise hindamise keerukaks.

Uuringu eesmärk

Antud olukorras, kus uuringute tulemused atmosfääri suurenenud [CO_2] mõjust isopreeni eritamisele on vastuolulised ning tegelik olukord ebaselge, on oluline siduda uuringud taimede ontogeneesi etappidega, lehtede pindala kasvuga võras ja taimede füsioloogilise kohastumisega.

Käesoleva väitekirja peamine eesmärk oli uurida isopreeni eritamise muutumist taime kasvamisel ning õhu suurenenud [CO_2] mõju sellele protsessile.

Täpsemalt olid töö eesmärgid järgmised.

1) Kontrollida hüpoteesi, kas CO_2 suurenenud sisaldus ergutab lehe pindala kasvu, mille tõttu võra lehtedest eritatav isopreeni hulk kasvab; uurida suurenenud [CO_2] mõju taime ontogeeneesile, lehtede suuruse muutust ning fotosünteesi ja isopreeni eritamise dünaamikat hübriidhaava võras.
2) Eristada suurenenud [CO_2] lühi- ja pikaajalist mõju isopreeni eritamisele. Kontrollida hüpoteesi, kas CO_2 suurenenud sisaldusega keskkonnas kasvamine mõjutab taime vastuseid, mida ta annab isopreeni eritamise teel keskkonnatingimuste muutumisele nii lühiajaliselt (hetkeline vastus) kui ka pika aja vältel (pikaajaline kohastumime).

3) Eristada substraadiks oleva DMADP ja isopreeni süntaasi aktiivsuse kontrollivat mõju isopreeni eritamisele taimedes, mis on kasvanud suurenenud [CO_2] juures. Kontrollida hüpoteesi, kas DMADP hulga võimalik varieerumine muudab isopreeni eritamise kaudu taime reaktsioone (vastuseid) ka teistele keskkonnateguritele nagu näiteks valgus, muutes selle kaudu valguse kasutamise efektiivsust isopreeni biosünteesil.
4) Saada ülevaade isopreeni võimalikust kaitsvast toimest kuuma vastu ja isopreeni eritamise regulatsioonidest kõrgete temperatuuride korral. Eriti tähtis on kontrollida hüpoteesi, kas õhu kõrgenenud [CO_2]-ga kohastunud taimed on kuumastressile vastupidavamad tänu kuumastressi enda põhjustatud suuremale isopreeni eritamisele.
5) Uurida keskkonna temperatuuri pikaajaliste muutuste ja lehestiku vananemisest tingitud muutuste mõju isopreeni eritamise võimele lehe vananemise jooksul.

Metoodika

(1) Eksperiment suurendatud [CO_2] mõjust isopreeni eritamisele.

Õhu suurendatud [CO_2] mõju uuringud isopreeni eritamise dünaamikale alates pungade võrsumisest kuni võra arenemiseni viidi läbi avatud gaasivahetussüsteemis. Eksperimenti valiti kahe-aastased hübriidhaavad (*Populus tremuloides* Michx. x *P. tremula* L.), kloon H55 ja H200, mis kasvasid normaalse väetamis- ja niiskusreziimiga kambrites. Avatud gaasivahetussüsteem, mis koosnes neljast eraldiseisvast klaaskambrist, konstrueeriti spetsiaalselt taimede kasvatamiseks ning nende gaasivahetuse aktiivsuse pikaajaliseks ja pidevaks jälgimiseks. Kaks täielikult taime ümbritsevat kambrit hoidsid CO_2 sisalduse 380 $\pm$ 10 μmol mol^{-1} juures (keskmine $\pm$ standardhälve), kahes kambris oli CO_2 sisaldus kõrgem - 780 $\pm$ 10 μmol mol^{-1}. Fotosünteesi ja isopreeni eritamise pidevaks jälgimiseks kombineeriti avatud gaasivahetussüsteemiga analüsaator LI-7000 CO_2/H_2O (Li-Cor Inc., Lincoln, NE, USA.) ja isopreeni kiirmõõtmise sensor (FIS, Hills-Scientific, Boulder, CO, USA). Kambrite temperatuuri hoiti 28-30/23°C juures vastavalt päeval ja öösel ning niiskust 60% juures. Iga kamber oli varustatud halogeenlambiga, mille valgusintensiivsus oli 800 $\mu mol^{-1}\ m^{-2} s^{-1}$ fotoperioodi (päeva ehk nähtava valguse aeg) kestusega 12 tundi.

(2) Eksperiment suurendatud [CO_2] tingimustes pikaajaliselt kasvanud (kohastunud) taimede isopreeni eritamise uurimiseks.

Pärast 5 nädalat, kui lehestik võras oli täielikult välja arenenud, võeti taimed avatud gaasivahetusega kambritest välja, et hinnata nende kohanemist suurendatud [CO_2]-ga ning selle mõju isopreeni eritamise ja fotosünteesi iseloomule, termilise stressi vastustele ning lehe anatoomia muutust. Taimi analüüsiti neljas erinevate [CO_2] tingimustega eksperimendis: 1) ümbritsevas õhus kasvanud taim ümbritseva õhu tingimustes - *ambient (380);* 2) ümbritsevas õhus kasvanud taim suurendatud [CO_2] tingimustes - *ambient (780);* 3) suurendatud [CO_2] tingimustes kasvanud taim ümbritseva õhu tingimustes - *elevated (380);* 4) suurendatud [CO_2] tingimustes kasvanud taim suurendatud [CO_2] keskkonnas - *elevated (780).*

(3) Eksperiment uurimaks taime isopreeni eritamise võime kohanemist vastavalt keskkonna muutustele sügisese lehtede vananemise käigus.

Isopreeni emissiooni muutuste sõltuvust keskkonna temperatuuri fluktuatsioonidest uuriti parasvöötme heitlehise puu, hariliku haava *(Populus tremula)* näitel. Mõõtmisi tehti lehtede vananemise jooksul hilissuvest kuni intensiivse lehtede langemise ajani. Hariliku haava (*Populus tremula* L.*)* võrsed lõigati vee all ja transporditi kohe laborisse, kus mõõdeti fotosünteesi iseloomu, isopreeni ja metanooli eritamist ning DMADP hulka.

Tulemused ja arutelu

(1) Suurenenud [CO_2] õhus mõjutab isopreeni emissiooni mitmel tasandil - lehe tasandist võra tasandini.

Hübriidhaava võra arengu dünaamika, CO_2 netoassimilatsioon võra lehestikus (*canopy net assimilation rate*) ja isopreeni eritamise kiirus (*isoprene emission rate*) sobis hästi kokku Chapman-Richerd'i funktsioonikõveraga, mis kirjeldab päevade lõikes toimuvaid protsesse. Suurenenud [CO_2] tulemusel kasvas võras lehtede maksimaalne pindala kuni 942 cm^2-ni, mis ületas ümbritsevas õhus kasvanud taime lehtede pindala (660 cm^2) 43%. Samuti kasvas CO_2 netoassimilatsioon võras ehk fotosünteesi intensiivsus

kiiremini ja saavutas kahekordse väärtuse (32 mmol päevas) võrreldes ümbritsevas õhus kasvanud puudega (16 mmol päevas). Suurendatud [CO_2] keskkonnas ulatus maksimaalne eritamisvoog (*emission flux*) võra lehestikust 38 μmol-ini päevas, mis on 1,4 korda suurem, kui ümbritsevas õhus kasvanud taimedel (27 μmol-i päevas).

Lehe tasandil sõltus CO_2 netoassimilatsioon (*leaf net assimilation rate or net assimilatsion rate*) ja isopreeni eritamise määr mõlema [CO_2]-ga töötluse korral lehe arengu dünaamikast väljendudes graafikul ühe maksimumiga kõverana. CO_2 suurem sisaldus õhus viis oluliselt suurema koguse CO_2 assimilatsioonini ja suurema fotosünteesi intensiivsuseni, kuna samal ajal vähenes isopreeni eritamise kiirus vähenes oluliselt.

Nii võra kui ka lehe tasandil sõltuvad mudeli parameetrid suurel määral CO_2 sisaldusest. Eksperimendi tulemus näitas, et võra tasandil toimuv täiendab oluliselt lehe tasandi protsesse ja isopreeni eritamine võib suurenenud [CO_2] juures tegelikult suureneda, kuna lehtede kogupindala kasvab.

(2) Suurenenud [CO_2]-ga õhus pikaajaline kasvamine muutis lehe iseloomulikke tunnuseid.

Pärast 5 nädalat kasvamist avatud gaasivahetussüsteemis, oli suurenenud [CO_2] mõjutanud oluliselt lehe anatoomilist struktuuri: paksem leht ja mesofüll, suurem kloropastide arv raku pinna kohta ning suuremad tärkliseterad kloroplastis viitasid suuremale fotosüneesiva koe hulgale ning suurema hulga fotosünteesi saaduste tekkimisele. Suurenenud [CO_2] ilmselt suurendas süsiniku ja lämmastiku sidumist ning muutis süsiniku ja lämmasiku suhet ($R_{C:N}$), mis näitas, et pika aja jooksul antud keskkonnas kasvanud taimed olid sellega kohastunud. Sellega kaasnes lehtede iseloomulike tunnuste muutumine ja fotosünteesiva koe areng.

(3) Suurenenud [CO_2]-ga õhus pikka aega kasvanud taimedel muutus DMADP hulk ja ISPS-i aktiivsus (fotosünteesi ja isopreeni sünteesi iseloom).

Pärast 5 nädalat suurenenud [CO_2]-ga keskkonnas kasvamist uuriti katsetaimedel lehe CO_2 netoassimilatsiooni, DMADP hulka ja ISPSi aktiivsust. Mõõtmistingimused langesid kokku kasvukeskkonna keskmise valguse intensiivsuse ja temperatuuri väärtustega. Suurenenud [CO_2]

tingimustes kasvanud taimedel oli CO_2 netoassimilatsioon suurem kui ümbritseva õhu tingimustes kasvanud taimedel ning isopreeni eritamise kiirus langes vähesel määral. CO_2 sisalduse tõus vähendas oluliselt DMADP hulka, kuna ISPS aktiivsus suurenes. Tulemused näitavad, et suurenenud [CO_2] juures kasvamine põhjustab taime kloroplastides kahe võtmeteguri, DMADP hulga ja ISPSi aktiivsuse muutuse, mille tõttu võib muutuda ka isopreeni eritamise iseloom.

(4) Kasvamine suurenenud [CO_2]-ga õhus muutis fotosünteesi ja isopreeni emissiooni näitajaid sõltuvalt valgusest ja CO_2-st.

Fotosünteesi ja isopreeni eritamise iseloomulikke tunnuseid uuriti nende reaktsioonide sõltuvuse kaudu valgusest ja CO_2 sisaldusest. Mõõtmistingimused langesid kokku kasvukeskkonna tingimustega - keskmise valguse intensiivsuse ja temperatuuri väärtustega. Suurenenud [CO_2]-ga õhus kasvamise tulemusel oli katsetaimedel oluliselt suurem ribuloos-1,5-bisfosfaadi karboksülaasi oksügenaasi (RuBisCO) aktiivsus (V_{cmax}), samuti kasvas fotosünteesi elektrontranspordi võime (J_{max}), CO_2 netoassimilatsioon (A_{max}) valgusküllastuse tingimustes, algne CO_2 kvantsaagis (Φ) ja algne fotosünteesi elektrontranspordi kvantsaagis (Φ_J).

Isopreeni eritamise (I) ja rakkude vahelise CO_2 sisalduse (C_i) sõltuvust väljendavad kõverad on asümmeetrilised, eristuva tipuga ning suuresti mõjutatud [CO_2]-st taimede kasvukeskkonnas. Suurenenud [CO_2]-ga keskkonnas kasvanud taimedel mõõdeti kõrgem isopreeni eritamise kiirus ($I_{Ci,max}$) ja vastavalt madalam $C_{i,max.}$ Isopreeni – valguse sõltuvuse graafikud näitavad, et suureneud [CO_2] keskkonnas kasvamise tulemusel suurenes isopreeni eritamine valgusküllastuse tingimustes ($I_{max,Q}$). Kuigi suurenenud [CO_2] keskkonnas kasvanud taimedel mõõdeti isopreeni eritamisel madalm kvantsaagis, mis näitab madalamat fotosünteesi elektrontranspordi efektiivsust.

Need uuringud näitasid, et pikaajaline suurenenud [CO_2]-ga keskkonnas kasvamine muudab lehestiku füsioloogilisi protsesse, samuti fotosünteesi kui ka isopreeni eritamise näitajaid. Suurenenud [CO_2]-ga keskkonnas kasvanud katsetaimedel kujunes välja suurem fotosünteesivõime, isopreeni eritamise võime ja suurem valguse kasutamise efektiivsus.

(5) Suurenenud [CO_2]-ga õhus pikaajaline kasvamine suurendab taimede stressitaluvust.

Suurenenud [CO_2]-ga õhus kasvanud taimedel oli oluliselt madalam üleminekufaasi (*phase-transition*) temperatuur puhul kõrgem kuumastressi puhul. Need taimed säilitasid nii 25°C kui ka 50°C temperatuuri juures madalama elektrilise mahtuvuse, kusjuures ümbritseva õhu tingimustes kasvanud taimedel tõusis nimetatud näitaja oluliselt. Kirjeldatud mõju viitab sellele, et suurenenud [CO_2]-ga keskkonnas kasvanud taimedel on suurem vastupidavus stressile tänu rakumembraani väiksemale läbilaskvusele.

Kui temperatuur tõusis 30°C-st kuni 50°C-ni, siis CO_2 netoassimilatsioon (*net assimilation rate*) langes, kuid isopreeni eritamise kiirus tõusis kõigil uuritud juhtudel (loetletud "Metoodika" alapeatükis, punktis 2). Tulemus peegeldab seda, et isopreeni eritamine muutub kuumastressi tingimustes fotosüsnteesist sõltumatuks. Kovariatsioonianalüüs (ANCOVA) näitas, et eksisteerib lineaarne seos CO_2 netoassimilatsiooni vähenemise ja isopreeni eritamise kiiruse suurenemise vahel. Tulemused näitavad, et suurenenud [CO_2]-ga keskkonnas kasvanud taimedel on stressi tingimustes vähenenud fotosünteesiprotsessis kõrgem kaitsevõime.

Huvitav tulemus on see, et temperatuuri tõusuga kaasnes lühiajaliselt suurenenud [CO_2] pärssiva mõju kadumine isopreeni eritamisele ja isopreeni eritamise kiirus sõltus peamiselt [CO_2] suurenemisest. See võib peegeldada isopreeni tekke regulatsiooni stressi käigus, mis on seotud vana või ladustatud süsiniku (tärklis ja suhkrud) mobiliseerumisega.

(6) Keskkonna temperatuur ja lehestiku vananemine põhjustavad muutusi isopreeni eritamises.

Keskkonna temperatuuri kasvu aeglustumise ja lehestiku vananemisega kaasnes fotosünteesi intensiivsuse (*photosynthetic rate*), isopreeni eritamise kiiruse ja DMADP hulga järsk vähenemine. Kuigi, fotosünteesi intensiivsuse ja isopreeni eritamise kiiruse vähenemine seostus peamiselt lehe lämmastiku sisalduse vähenemisega. Tulemus näitab, et ka vananevate lehtede võime eritada isopreeni võib kohaneda keskkonnatingimustega, kuid nende lehtede reaktsioon muutustele on nõrk võrreldes vegetatsiooni kõrgpunkti-aegsega, ja see on mõjutatud ka stressist.

Kokkuvõte

Käesoleva väitekirja raames tehtud uuringud näitasid, et isopreeni eritamine on mõjutatud taime ontogeneesist ja erinevatest keskkonnateguritest. Suurenenud [CO_2] mõju hübriidhaava isopreeni eritamisele oli võra ja lehe tasandil erinev. Võra lehestikus oli see suurenenud [CO_2]-ga õhus kasvanud taimedel suurem kui ümbritsevas õhus kasvanud taimedel. Selle põhjuseks oli suurenenud [CO_2] mõjul lehestiku pindala suurenemine, mis kompenseeris lehe tasandil toimunud isopreeni eritamise vähenemise. Samuti muutusid hübriidhaava lehele iseloomulikud tunnused, tõusis fotosünteesi ja isopreeni eritamise võime. Koos isopreeni eritamise kiiruse tõusuga paranes hübriidhaava termotolerants. Isopreeni eritamise iseloomulikud jooned harilikul haaval seostusid looduslikul lehtede kolletamisajal (vananemisperioodil) keskkonna temperatuuriga ja lehestiku vananemisest tingitud eneseregulatsiooniga.

Suurenenud [CO_2] mõju taimele on mitmetine ja kohastumisefekt võib olla seotud suurema vastupanuvõimega stressile. Seetõttu tuleb isopreeni eritamise mudelite loomisel, mis ennustaksid tuleviku olukorda atmosfääri suurenenud [CO_2] tingimustes, arvestada taime kohastumist ontogeneesi käigus, tema stressitaluvust ning lehe ja lehestiku tasandil toimuvate protsesside dünaamikat.

Väitekirjas esitatu põhjal saab soovitada, et vajalik oleks läbi viia suures ulatuses uuringud vaba õhu CO_2-ga rikastumise (*free air enrichment – FACE*) seireks, et jälgida võras lehestiku tasandi tunnuseid (lehepinna indeks, biomass), mille muutumisega taim reageerib CO_2 suurenenud sisaldusele, ja mõõta isopreeni eritamist. Töös kirjeldatud eksperimendid toimusid mõõdukalt muudetud tingimustes ja hea väetamise juures, mis arvestas toitainete kättesaadavust nii lehestiku morfoloogilise aregu vältel *("morphological upregulation"*) kui ka fotosünteesi pidurdumisel *("downreglation of photosunthesis"*). Samuti soovitame edasistes uuringutes kasutada suurendatud [CO_2]-ga eksperimentides taimede väetamist erinevate toitainete kogustega.

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I

PUBLICATIONS

Research

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Elevated atmospheric CO_2 concentration leads to increased whole-plant isoprene emission in hybrid aspen (*Populus tremula* × *Populus tremuloides*)

Zhihong Sun[1], Ülo Niinemets[1], Katja Hüve[1], Bahtijor Rasulov[2] and Steffen M. Noe[1]

[1]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu, 51014, Estonia; [2]Institute of Molecular and Cell Biology, University of Tartu, Riia 23, Tartu, 510101, Estonia

Author for correspondence:
Steffen M. Noe
Tel: +372 59 192254
Email: steffen.noe@emu.ee

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Summary

- Effects of elevated atmospheric [CO_2] on plant isoprene emissions are controversial. Relying on leaf-scale measurements, most models simulating isoprene emissions in future higher [CO_2] atmospheres suggest reduced emission fluxes. However, combined effects of elevated [CO_2] on leaf area growth, net assimilation and isoprene emission rates have rarely been studied on the canopy scale, but stimulation of leaf area growth may largely compensate for possible [CO_2] inhibition reported at the leaf scale. This study tests the hypothesis that stimulated leaf area growth leads to increased canopy isoprene emission rates.
- We studied the dynamics of canopy growth, and net assimilation and isoprene emission rates in hybrid aspen (*Populus tremula* × *Populus tremuloides*) grown under 380 and 780 μmol mol^{-1} [CO_2]. A theoretical framework based on the Chapman–Richards function to model canopy growth and numerically compare the growth dynamics among ambient and elevated atmospheric [CO_2]-grown plants was developed.
- Plants grown under elevated [CO_2] had higher C : N ratio, and greater total leaf area, and canopy net assimilation and isoprene emission rates. During ontogeny, these key canopy characteristics developed faster and stabilized earlier under elevated [CO_2]. However, on a leaf area basis, foliage physiological traits remained in a transient state over the whole experiment.
- These results demonstrate that canopy-scale dynamics importantly complements the leaf-scale processes, and that isoprene emissions may actually increase under higher [CO_2] as a result of enhanced leaf area production.

Introduction

The majority of biogenic volatile organic compounds (BVOCs) are emitted from terrestrial sources such as forests, grasslands, shrublands and croplands (Guenther *et al.*, 1995; Peñuelas & Staudt, 2010). Isoprene is among the most abundant BVOCs emitted from vegetation (Sharkey & Yeh, 2001; Sharkey *et al.*, 2008). Its share may reach up to 40% of total BVOC emissions, with estimated yearly totals of 440–660 Tg C yr^{-1} (Guenther *et al.*, 2006). Previous studies have revealed that isoprene plays an important physiological role in protecting plants from biotic and abiotic stresses (Sharkey & Singsaas, 1995; Behnke *et al.*, 2007; Loivamäki *et al.*, 2008; Vickers *et al.*, 2009; Velikova *et al.*, 2011). In particular, dissipation of excess energy to protect the photosynthetic apparatus (Sanadze, 2010), stabilizing thylakoid membranes at high temperatures (Sharkey & Singsaas, 1995; Singsaas & Sharkey, 1998, 2000; Owen & Peñuelas, 2005; Behnke *et al.*, 2007; Velikova *et al.*, 2011), quenching of reactive oxygen species (Affek & Yakir, 2002; Sharkey *et al.*, 2008; Vickers *et al.*, 2009), and repelling herbivores (Loivamäki *et al.*, 2008) have been reported.

Isoprene also plays a major role in tropospheric photochemistry and contributes to secondary organic aerosol formation, thereby potentially influencing large-scale Earth system processes (Fehsenfeld *et al.*, 1992; Claeys *et al.*, 2004; Hallquist *et al.*, 2009). As a very reactive volatile compound, it strongly affects ozone and secondary organic aerosol formation in the troposphere (Williams *et al.*, 1997; Fuentes *et al.*, 2000; Kroll *et al.*, 2005) and partly controls the lifetime of the glasshouse gas methane by its reaction with hydroxyl radicals (Kaplan *et al.*, 2006).

Isoprene is formed in chloroplasts by isoprene synthase from its immediate precursor dimethylallyldiphosphate (DMADP) via the 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway (Lichtenthaler *et al.*, 1997) and a major part of its carbon skeleton is derived from recently assimilated photosynthates (Lichtenthaler, 1999; Affek & Yakir, 2003; Trowbridge *et al.*, 2012). Thus, DMADP availability and isoprene synthase activity are key factors determining the isoprene emission rate (Calfapietra *et al.*, 2008; Rasulov *et al.*,

2009, 2010; Li *et al.*, 2011), although the physiological regulation mechanisms of isoprene synthesis have still not been fully resolved. The instantaneous isoprene emission rate is strongly light- and temperature-dependent and this response is similar for different plant species. The instantaneous responses result from changes in the supply of intermediates to isoprene synthesis (Loreto & Sharkey, 1993; Schnitzler *et al.*, 2004; Magel *et al.*, 2006; Rasulov *et al.*, 2009, 2010).

Over the long term, prevailing environmental conditions and leaf ontogeny affect the development of isoprene synthesis capacity (Kuzma & Fall, 1993; Sasaki *et al.*, 2005; Loivamäki *et al.*, 2007; Cinege *et al.*, 2009; Niinemets *et al.*, 2010a; Sun *et al.*, 2012a). The isoprene emission capacity starts to develop just before full leaf photosynthetic competence, a pattern observed in velvet bean (*Mucuna* sp.; Kuzma & Fall, 1993; Harley *et al.*, 1994) and aspen (*Populus tremuloides*; Monson *et al.*, 1994). After reaching a maximum isoprene emission rate, the isoprene emission capacity starts to decline in senescing leaves (Kuhn *et al.*, 2004; Sun *et al.*, 2012a). These modifications are associated with changes in isoprene synthase gene expression and isoprene synthase protein content (Mayrhofer *et al.*, 2005). Although isoprene synthase gene is 'constitutively' expressed, its promoter region contains circadian-, heat-, and stress-dependent elements, and the promoter activity depends on light and temperature over days to weeks (Loivamäki *et al.*, 2007; Cinege *et al.*, 2009).

A further important, and much less understood, driver that affects short- and long-term isoprene emissions is ambient [CO_2]. Effects of growth [CO_2] on isoprene emissions have been studied in different plant species under various experimental conditions with controversial outcomes. Elevated [CO_2] had no or only a moderate effect on the isoprene emission capacity in *Populus alba* (Loreto & Velikova, 2001; Loreto *et al.*, 2007), *P. tremuloides* (Calfapietra *et al.*, 2008), and *Populus* × *euramericana* (Centritto *et al.*, 2004), while elevated [CO_2] resulted in enhanced isoprene emission capacity in *Quercus rubra* (Sharkey *et al.*, 1991), *Quercus pubescens* (Tognetti *et al.*, 1998), *Gingko biloba* (Li *et al.*, 2009) and *Populus tremula* × *P. tremuloides* (Sun *et al.*, 2012b). In other studies, elevated [CO_2] led to a remarkable depression of isoprene emissions, including *P. deltoides* (Rosenstiel *et al.*, 2003), *Acacia nigrescens* (Possell & Hewitt, 2011), *Liquidambar styraciflua* (Monson *et al.*, 2007; Wilkinson *et al.*, 2009), *Populus tremuloides* (Sharkey *et al.*, 1991; Darbah *et al.*, 2010), *Eucalyptus globulus*, *P. tremuloides* and *P. deltoides* (Wilkinson *et al.*, 2009), *Phragmites australis* (Scholefield *et al.*, 2004), and *Platanus orientalis* (Velikova *et al.*, 2009). However, most studies on the inhibition of isoprene emission by elevated [CO_2] were carried out at the leaf level and described mostly the response to instantaneously elevated [CO_2], thereby mixing up the instantaneous CO_2 response and the long-term acclimation response (see Sun *et al.*, 2012b for a detailed discussion). In fact, the effects of elevated [CO_2] on plants are multifaceted, involving instantaneous and acclimation metabolic responses at the leaf scale, and whole-plant processes such as acceleration of plant and leaf growth rates, leading to faster biomass accumulation, but also to alterations in stand development dynamics (Gielen *et al.*, 2003; Rapparini *et al.*, 2004; Arneth *et al.*, 2007; Liberloo *et al.*, 2007; Monson *et al.*, 2007; Niinemets, 2010b). For constructing predictive models of isoprene emission under higher atmospheric [CO_2], it is essential to consider plant acclimation and ontogeny.

Atmospheric [CO_2] has been rising since the industrial revolution (Long *et al.*, 2004; Rapparini *et al.*, 2004; IPCC, 2007) and is predicted to continue to rise and to affect the global climate (Fuentes *et al.*, 2000; Wiedinmyer *et al.*, 2006). Yet, many models that predict isoprene emission from plants are based on empirical or semi-mechanistic algorithms (Guenther *et al.*, 1993, 2006; Niinemets *et al.*, 1999; Heald *et al.*, 2009). These models usually utilize leaf-scale measurements and rely on meteorological input parameters as driving factors. To account for the effects of elevated [CO_2], the models typically use an empirical parameterization based on measurements of instantaneous enhancements of [CO_2] (Wilkinson *et al.*, 2009). In several studies, it has been speculated that a possible increase in leaf area might cancel out the declining effect of instantaneous [CO_2] elevation on isoprene emission (Rosenstiel *et al.*, 2003; Centritto *et al.*, 2004; Sun *et al.*, 2012b). To our knowledge, this hypothesis has been tested with dense poplar stands at midseason when stand leaf area was the highest; in this study, leaf area increase at higher [CO_2] moderated the leaf-level [CO_2] effect by 15–50%, but did not fully offset the effect of reduced isoprene emission at leaf scale (Rosenstiel *et al.*, 2003). However, at the canopy scale, the situation becomes further complicated by enhanced shading by increasing leaf area that might also reduce isoprene emission (see earlier). Thus, the stand-scale effect can strongly depend on ontogenetic characteristics. In rapidly developing more open stands, elevated [CO_2] effects on leaf area can be more important than in fully closed stands exhibiting a steady-state leaf area index (LAI). Thus, for fast-growing stands, it is important to monitor the stand-level [CO_2] response through the start of canopy development to closure.

In this study, we investigated carbon assimilation and isoprene emission in hybrid aspen (*P. tremuloides* × *P. tremula*) on canopy and leaf level from the start of canopy development to maturation under different ambient [CO_2]. Our main aim was to test the hypothesis that the canopy isoprene emission of hybrid aspen grown under elevated [CO_2] is increased even though an instantaneous effect of elevated [CO_2] lowers isoprene emission at the level of individual leaves. We have previously demonstrated that growth under elevated [CO_2] did not affect isoprene emissions when gauged under the same CO_2 concentration (either ambient or elevated) at moderate-high light intensity (Sun *et al.*, 2012b). Here we further use a modeling framework to quantitatively analyze the dynamics of canopy development among plants grown under current ambient and elevated [CO_2].

Materials and Methods

Plant material and growth system

Two-year-old saplings of hybrid aspen (*Populus tremuloides* Michx. × *Populus tremula* L.) clone H55 were selected for the

experiments (Oksanen *et al.*, 2001). The clone is a cross between a female *P. tremula* L. of Finnish origin and a male *P. tremuloides* Michx. of Canadian origin (Häikiö *et al.*, 2009). H55 is widely used in Estonian and Finnish forestry as a fast-growing hybrid with moderate tolerance to ozone. The selected plants were *c.* 0.2 m tall and kept at −2°C in a dormant state before the experiments. Dormancy was broken by placing the plants in a growth room at 20°C 4 d before the start of the experiment. The saplings with swelling buds were potted in plastic pots (diameter 0.2 m, height 0.2 m) filled with 1 kg of sand and peat mixture (1 : 1) and installed in the open gas-exchange system consisting of four glass chambers of 12.5 l volume (diameter 0.2 m, height 0.4 m). Bud opening and leaf development took place inside the chambers. The plants were watered daily with tap water until the soil reached field capacity. To ensure optimum nutritional supply, the pots where initially fertilized with a slow-release fertilizer, and a liquid fertilizer was applied twice during the growth period as described in Sun *et al.* (2012b).

The glass chambers were connected to a gas-exchange system, allowing for continuous measurements of net assimilation, transpiration and isoprene emission rates (see Sun *et al.*, 2012b for a detailed description of the system). Chambers 1 and 3 (Fig. 1) were kept at a CO_2 concentration (average ± SD) of 380 ± 10 μmol mol^{-1} (hereafter denoted as ambient), while chambers 2 and 4 were treated with an elevated CO_2 concentration of 780 ± 10 μmol mol^{-1} (hereafter denoted as elevated). Environmental conditions in the chambers were set for a 12 h photoperiod as described by Sun *et al.* (2012b). Temperature was maintained at 28–30: 23°C for day: night conditions, relative humidity at 60%, and light intensity at 800 μmol m^{-2} s^{-1} measured directly below the top boundary of the chambers. We repeated the experiment five times with 20 plants in total, 10 for ambient [CO_2] and 10 for elevated [CO_2] conditions.

Plant canopy leaf area estimation during the experiment

Leaf area growth during the experiment was assessed by a method combining digital photography and destructive harvesting. The plants in the chambers were photographed daily at a fixed time and from exactly the same position. The silhouette of the leaf area on each photograph was determined using GIMP (The GNU Image Manipulation Program, Version 2.6, www.gimp.org) by removing the background, the stem and petioles. During the first 1–3 d, the plant leaves were very small, and the 'leaf area' was defined as the area of swelled or partly opened buds. Within 30–40 d after the start of the experiment, the plants developed a canopy that filled the whole chamber volume. At that point, the experiment was stopped, the plants were removed, and all leaf blades were harvested and subsequently scanned to assess the total canopy leaf area at the end of each experimental run. In addition, we used data from 28 additional plants for calibration of digital photography. In these plants, we monitored the growth of all the individual leaves at daily intervals by tracing the outline of the leaves on paper and also taking digital photographs. The plants with different amounts of leaf area were harvested between 1 and 30 d after the start of the experiment. Based on these data, we developed a linear regression model relating the photographic silhouette leaf area and the scanned leaf area (Fig. 2). Mathematica 8 (Mathematica; Wolfram Research Inc., Champaign, IL, USA) was used to fit the data by a least-squares method. Estimating the projected leaf area from the silhouette leaf area may lead to a bias if the foliage aggregation varies during the experiment and/or among the treatments (Cescatti & Niinemets, 2004). Such variations in the degree of aggregation are expected to lead to curvilinearity or scatter in the regressions. However, in our study, there was no evidence of curvilinearity, and the relationships were strong when all data were pooled, suggesting that possible variations in the degree of spatial aggregation did not play a role in our study.

Online canopy net assimilation and isoprene emission measurements

The gas stream for the four parallel open gas-exchange chambers (see Fig. 1) was divided between the reference flow (the air entering the chamber) and the sample flow (the gas leaving the plant chamber). The analyzer ports were switched between the reference and sample modes, sequentially sampling the different

Fig. 1 Photograph of the computer-controlled growth chamber and gas exchange system used to control the ambient [CO_2] during canopy development.

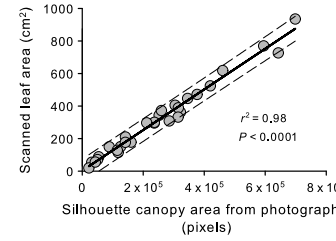

Fig. 2 Calibration of the hybrid aspen (*Populus tremula* × *Populus tremuloides*) plant canopy leaf area by linear regression of photographically estimated foliage silhouette area (*x*-axis) and the scanned whole-plant true projected leaf area (*y*-axis) for plants of different ages. The regression equation is $y = 0.0012x + 2.17$. The dashed lines denote the 95% confidence intervals for the mean.

chambers. Reference and sample flow gas concentrations were measured separately with an LI-7000 CO_2/H_2O analyzer (Li-Cor Inc., Lincoln, NE, USA) and a fast isoprene sensor (FIS; Hills-Scientific, Boulder, CO, USA). Each single chamber measurement lasted for 120 s (60 s for reference and 60 s for chamber flow). Thus one measurement cycle over four chambers lasted for 8 min. Isoprene concentration was recorded in 5 s intervals and CO_2 assimilation in 30 s intervals. The FIS operates on the principle of chemiluminescence reaction between isoprene and ozone (as described in Monson *et al.*, 1991; Zimmer *et al.*, 2000; Pegoraro *et al.*, 2005, 2006). The analyzer was calibrated frequently with a gas standard containing 5.74 ppm isoprene in N_2, and operated as described previously (Rasulov *et al.*, 2009).

From the reference and sample CO_2 and isoprene concentrations, instantaneous canopy net assimilation (A_C) and isoprene emission (I_C) rates were obtained (Fig. 3a,b). By integrating the instantaneous rates, daily integrated canopy net assimilation (CO_2, $A_{C,day}$) and isoprene emission ($I_{C,day}$) rates were calculated (Fig. 3a,b). In addition, net assimilation (A) and isoprene emission rates (I) per unit leaf area were calculated by dividing the whole-plant rates by the leaf area estimates for the given day (see the section, Modeling net assimilation and isoprene emission rates on the leaf scale).

A dynamic model of canopy leaf area development, canopy assimilation rate and isoprene emission rate

Plant growth models have been developed for many different purposes. Generally, these models intend to describe the growth state of the whole plant, its organs or physiological processes in one life cycle (Yin *et al.*, 2003). In classical plant growth analysis, a simple exponential growth model is commonly used (Evans, 1972; Causton & Venus, 1981; Hunt, 1982). However, the exponential model is only valid for the initial period of plant growth. The growth rate gradually slows down as plants accumulate nonphotosynthetic tissue and leaf area, resulting in greater respiration rate and higher self-shading (Evans, 1972; Causton & Venus, 1981; Hunt, 1982). Analogously, the rate of physiological processes levels off with increasing plant size (Coleman *et al.*, 1993; McConnaughay & Coleman, 1999). To simulate the entire plant growth time series, several empirical growth models have been suggested. The Chapman–Richards function (Bertalanffy, 1957; Evans, 1972; Pienaar & Turnbull, 1973; Causton & Venus, 1981; Hunt, 1982; Liu & Li, 2003) is a widely used growth model which is based on the assumption that both the plants' physiological state and the environment affect the growth pattern. Assuming that positive assimilation or accumulation and negative dissimilation or consumption processes occur, the change of growth over time may be expressed as (Bertalanffy, 1957; Pienaar & Turnbull, 1973):

$$\frac{dy}{dt} = \alpha y^m - \beta y \qquad \text{Eqn 1}$$

where y denotes the size of the growing element (population, individual, organ) or changing process rate, α represents a positive and β a negative metabolic factor. The parameter m modulates the positive growth term and is usually related to environmental influences. After integration and application of the parameter transformations given by Eqns S2–S4 (in Supporting Information Methods S1), we can rewrite Eqn 1 to:

$$y(t) = y_0 + \lambda(1 - e^{-rt})^c \qquad \text{Eqn 2}$$

where $y(t)$ denotes the state of the measured variable at time t, y_0 is the size of the growing component or rate of the process at time $t = 0$, λ is the maximum increase of the growing resource, r is the relative growth rate, and c determines the curve shape. Even though the growth model (Eqn 2) is very flexible and can be fitted to different growth processes (Bertalanffy, 1957; Yin *et al.*, 2003), independent estimations of model parameters may not always converge. The main reason for that behavior is evident in Eqns S1–S4 (in Methods S1), as the parameters λ, r and c all depend on m, and this interdependence may lead to collinearity.

We solved for the second-order derivative of Eqn 2 (see Methods S1) to estimate the time of fastest growth change at the inflection point of the curve and to determine the corresponding pair of function and argument values (y_i, t_i) as well as the maximum process rate (R_i). The model and parameters were used to fit the time-dependent changes in canopy leaf area, canopy net assimilation rate and canopy isoprene emission rate during the experiment.

Eqn 2 was used to describe the plant canopy leaf area development over time (see Fig. 4), time-dependent changes in daily

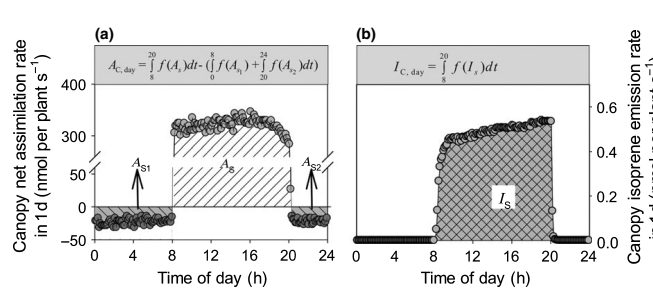

Fig. 3 Examples of diurnal variations in canopy net assimilation rate (a) and isoprene emission rate (b) in hybrid aspen (*Populus tremula* × *Populus tremuloides*). The data is stored in 5 s intervals and the daily integrated values are calculated according to the equations given in the figure.

integrated canopy net assimilation rate ($A_{C,day}$) and isoprene emission rate ($I_{C,day}$), and key parameters describing the dynamics of these canopy-level processes were derived using Mathematica 8 by a least-squares method.

Modeling net assimilation and isoprene emission rates on the leaf scale

Typically on a leaf scale, net assimilation and isoprene emission rate are given as fluxes (exchange rates per unit leaf area). We used the canopy-scale assimilation rate and isoprene emission rate divided by the leaf area in a given day to obtain the leaf-level fluxes. Canopy growth leads to shading of leaves within the canopy. Shading leads to changed environmental conditions in terms of light availability within the canopy and thus needs to be accounted for. Here, we have assumed that Eqn 2 remains valid and the loss of light energy through the canopy is caused by shading. We simulated the changes in light transmission arising as a result of time-dependent changes in canopy density according to the Lambert–Beer law (see Methods S1). Multiplication of Eqn S10 (in Methods S1) by Eqn 2 yields the leaf-level process rate as:

$$z(t) = z_0 + \left(-\log_e\left(\frac{Q(t)}{Q_0}\right) \cdot \lambda (1 - e^{-rt})^c \right) \qquad \text{Eqn 3}$$

We fixed the parameter Q_0 to 400 μmol quanta $m^{-2}\,s^{-1}$, which reflects the quantum flux density at half-height of the chamber at the time of plant installation in the chamber. The empirical parameter $Q(t)$ is the transmitted quantum flux density at time t and z_0 is the offset parameter (see Methods S1 for further details).

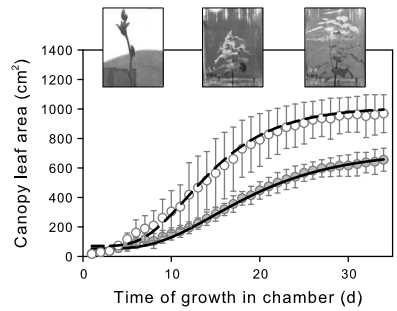

Fig. 4 Expansion of the hybrid aspen (*Populus tremula* × *Populus tremuloides*) plant canopy leaf area under ambient [CO_2] of 380 μmol mol^{-1} (gray circles) and elevated [CO_2] of 780 μmol mol^{-1} (white circles) over 35 d. The data are given as circles with error bars denoting the standard deviations (means ± SD). The solid line denotes the ambient [CO_2] and the dashed line the elevated [CO_2] modeled canopy leaf area as fitted by nonlinear least-squares regressions to Eqn 2 ($r^2 > 0.98$, $P < 0.0001$).

Leaf structural and chemical analyses

After the measurements were completed, the trees from each of the two treatments were harvested for structural and chemical analyses. Whole-canopy leaf area and fresh mass were estimated immediately after harvesting and leaf dry mass after drying at 70°C for at least 48 h. Foliage nitrogen (N_M) and carbon content (C_M) per unit dry mass were measured with a Vario MAX CNS analyzer (Elementar Analysen Systeme GmbH, Hanau, Germany). Finally, leaf dry : fresh mass ratio (D_F), leaf dry mass per unit area (M_A), leaf nitrogen (N_A) and carbon content (C_A) per unit leaf area were calculated.

Data analysis

Average whole-plant leaf morphological and chemical data and Eqn 2 model parameters for canopy- and leaf-level traits were compared among the treatments, elevated vs ambient, by paired *t*-tests with SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Normality of the variables was always tested by Kolmogorov–Smirnov test. Altogether, 10 replicate estimates for every characteristic were available for both treatments. All statistical relationships were considered significant at $P < 0.05$.

Results

Effects of elevated CO_2 concentration on canopy leaf area development and foliage traits

Elevated [CO_2] significantly increased canopy leaf area (Fig. 4), whole-canopy leaf dry mass (M_C) and dry mass per unit leaf area (M_A; Table 1) as compared with these traits in plants grown under ambient [CO_2]. Carbon content per unit dry mass (C_M) was not affected by the CO_2 concentration during leaf growth, while carbon content per unit leaf area (C_A) was increased significantly. Nitrogen content per unit dry mass (N_M) was somewhat lower under elevated [CO_2], and the C : N ratio was greater at elevated [CO_2] (Table 1).

In our experiments, the onset of photosynthesis was observed within 2–4 d after plant installation in the growth chamber. The Chapman–Richards function fitted the temporal variation of canopy leaf area growth with high degree of explained variance (Eqn 2 and Fig. 4, $r^2 > 0.98$, $P < 0.0001$). The predicted asymptotic canopy leaf area was 43% greater under elevated [CO_2] (942 cm^2) than under ambient [CO_2] (660 cm^2; Fig. 4, Table 2). For plants grown under elevated [CO_2], the relative leaf area growth rate was increased by 38% and the time of fastest growth, reached on the 12th day, was 2 d earlier than in the plants under ambient [CO_2] (Table 2).

Canopy net assimilation and isoprene emission rates

Continuous diel recordings (see Fig. 3 for representative diel variations in net assimilation and isoprene emission rates) were used to calculate the daily net assimilation and isoprene emission rates. On the canopy scale, both integrated daily net assimilation and

Table 1 Effects of growth [CO_2] environment on mean (± SE) leaf anatomical and chemical traits in hybrid aspen (*Populus tremula* × *Populus tremuloides*) leaves

Trait	Treatment		*P*-value†
	Ambient (380 μmol mol^{-1})	Elevated (780 μmol mol^{-1})	
Whole-canopy leaf dry mass (M_C, g per plant)	1.64 ± 0.21	2.64 ± 0.14*	0.003
Dry mass per unit area (M_A, g m^{-2})	28.5 ± 1.9	35.0 ± 1.5*	0.03
Carbon content per unit dry mass (C_M, %)	43.29 ± 0.33	43.53 ± 0.29	0.6
Carbon content per unit leaf area (C_A, g m^{-2})	12.4 ± 0.8	16.0 ± 1.1*	0.02
Nitrogen content per unit dry mass (N_M, %)	2.34 ± 0.24	1.66 ± 0.13*	0.03
Nitrogen content per unit leaf area (N_A, g m^{-2})	0.70 ± 0.070	0.64 ± 0.036	0.5
Carbon to nitrogen mass ratio	19.0 ± 1.70	26.2 ± 2.22*	0.03

Ten independent samples (trees) were available for each treatment.
*Significant differences between the means at $P < 0.05$.
†Means were compared by paired *t*-tests after testing for normality.

isoprene emission rates followed the dynamics of leaf area growth (Fig. 5), and Eqn 2 provided excellent fits to the temporal time-courses of both processes (Fig. 5, $r^2 > 0.98$, $P < 0.0001$). During the first 2–4 d, the plants respired until a sufficiently large leaf area was developed and leaves matured. The onset of isoprene emission was not substantially delayed as previously reported (Kuzma & Fall, 1993; Wiberley *et al.*, 2005) and started about 1 d later than whole-canopy photosynthesis became positive.

Transformation of the model parameters according to Eqns S6–S8 (in Methods S1) allows for estimation of relevant physiological characteristics of canopy maturation (Fig. 5, Table 3). Canopy net assimilation rate increased faster, but not earlier, under elevated [CO_2], and reached a maximum value of 32 mmol d^{-1}, almost double that under ambient [CO_2] (16 mmol d^{-1}; Fig. 5a, Table 3). The maximum slope (R_i) of the canopy net assimilation was found on the 12th day for both treatments (Table 3), but the plateau value (90% of maximum) was reached at day 22 in the case of elevated [CO_2] and day 33 in the case of ambient [CO_2]. On average, the plants under the elevated [CO_2] had 1.4 times higher relative daily growth rate than the plants under ambient conditions.

The maximum canopy isoprene emission rate was 1.4-fold higher for plants under elevated [CO_2] (38 vs 27 mol d^{-1}; Fig. 5b, Table 3). The relative growth rates of isoprene emission were not significantly different for both treatments but the time of fastest growth differed by 1 d: day 14 for elevated [CO_2] and day 15 for ambient [CO_2].

Leaf-scale net assimilation and isoprene emission fluxes

The process rates expressed per unit leaf area highlighted two important points. First, the isoprene emission flux was lower under elevated [CO_2] and, secondly, the shape of the developmental dynamics was changed and the curves exhibited a maximum. The decreases in flux rates beyond the maxima (Fig. 6) were assumed to be caused by increasing self-shading within the growing canopy. Thus, the data were better described by Eqn 3, which considered the increased shading during canopy expansion (Table 4). The maxima of net assimilation flux were observed at about day 18 in both treatments (Fig. 6), but elevated [CO_2]-grown plants had around a 1.4-fold higher maximum assimilation rate than ambient [CO_2]-grown plants (8.7 vs 6.1 mol m^{-2} s^{-1}; Fig. 6, Table 4).

Maximum isoprene emission flux was observed at day 25 under elevated [CO_2], and at day 23 under ambient [CO_2], and the maximum flux was 1.3-fold lower under elevated [CO_2]

Table 2 Hybrid aspen (*Populus tremula* × *Populus tremuloides*) canopy leaf area development parameters (means ± SE) according to the Chapman–Richards model (Eqn 2)

	y_0 (cm^2)	LA (cm^2)	r (d^{-1})	c	t_i (d)	L_i (cm^2)	R_i (cm^2 d^{-1})
Ambient	52 ± 8	660 ± 54	0.13 ± 0.011	6.5 ± 1.3	14 ± 1	220 ± 19	33.6 ± 3.2
Elevated	71 ± 6	942 ± 59*	0.18 ± 0.021*	8.2 ± 0.8*	12 ± 1	323 ± 20*	67 ± 9*
P-value†	0.191	< 0.0001	0.033	0.029	0.262	< 0.0001	0.016

The parameters are defined as: the offset (y_0), the maximal increase (LA), the relative growth rate (r) and the empirical parameter (c). The time (t_i) and leaf area (L_i) at the time of fastest growth as well as the maximal process rate (R_i) have been calculated according to Eqns S6–S8 (in Methods S1).
*Significant difference between the mean values at $P < 0.05$.
†Differences in the mean values ($n = 10$ for all parameters) were compared between the treatments by paired *t*-tests after normality was confirmed.

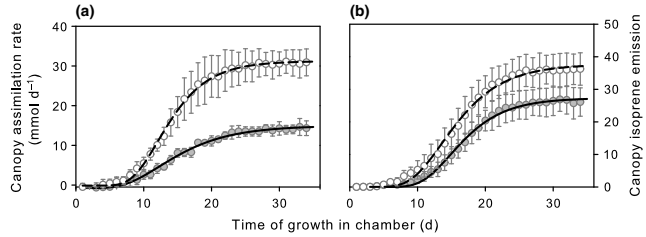

Fig. 5 Dynamics of measured and modeled hybrid aspen (*Populus tremula* × *Populus tremuloides*) canopy-scale physiological processes: daily net assimilation rate (a) and daily isoprene emission rate (b). Data are given as means ± SD. Gray circles, ambient[CO_2] treatments; white circles, elevated [CO_2] treatments. The lines denote nonlinear least-squares regressions to Eqn 2 ($r^2 > 0.98$, $P < 0.0001$), with the solid line representing ambient [CO_2] and the dashed line elevated [CO_2].

Table 3 Temporal dynamics of hybrid aspen (*Populus tremula* × *Populus tremuloides*) whole-canopy assimilation and isoprene emissions. Mean (± SE) parameters of canopy-scale daily net assimilation and isoprene emission rates fitted by Eqn 2

	y_0 (mmol d^{-1})	$A_{max,canopy}$ (mmol d^{-1})	r (d^{-1})	c	t_i (d)	A_i (mmol d^{-1})	R_i (mmol d^{-2})
Net assimilation							
Ambient	−0.951	16.0 ± 0.9	0.17 ± 0.017	9.7 ± 2.6	12 ± 1	5.36 ± 0.37	1.07 ± 0.07
Elevated	0.432	32.0 ± 0.9*	0.25 ± 0.035*	31 ± 9*	12 ± 1	11.33 ± 0.43*	3.07 ± 0.43*
P-value†	0.233	< 0.00001	0.021	0.032	0.684	< 0.00001	0.003
	y_0 (mmol d^{-1})	$I_{max,canopy}$ (μmol d^{-1})	r (d^{-1})	c	t_i (d)	I_i (μmol d^{-1})	R_i (μmol d^{-2})
Isoprene emission							
Ambient	0.000	27.2 ± 1.8	0.238 ± 0.012	39 ± 8	15 ± 1	9.8 ± 0.7	2.45 ± 0.26
Elevated	0.000	37.6 ± 2.2*	0.220 ± 0.014	23.2 ± 4.5*	14 ± 1	13.4 ± 0.8*	3.11 ± 0.23*
P-value†		0.002	0.326	0.16	0.386	0.003	0.031

$A_{max,canopy}$ denotes the maximal increase in daily assimilation rate, and $I_{max,canopy}$ denotes the isoprene emission rate; the other parameters (y_0, r, c) have the same meaning as in Table 2. Parameters for the time (t_i) and process value (A_i or I_i) at the point of fastest growth and the maximal process rate R_i were calculated as means ± SE according to Eqns S6–S8 (in Methods S1).

*Significant difference between the mean values at $P < 0.05$.

†Differences in the mean values ($n = 10$ for all parameters) were compared between the treatments by paired *t*-tests after normality was confirmed.

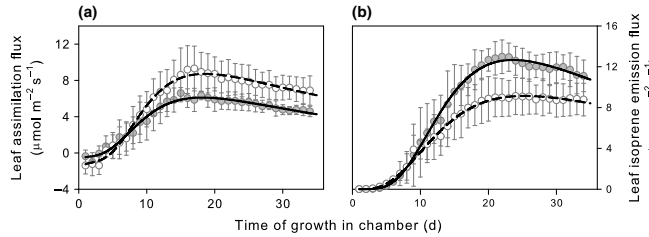

Fig. 6 Time courses of net assimilation flux (a) and isoprene emission flux (b) in hybrid aspen (*Populus tremula* × *Populus tremuloides*) rescaled to leaf scale for different [CO_2] treatments (gray circles, ambient [CO_2]; white circles, elevated [CO_2]). Data are given as means ± SD. The leaf-scale values were obtained from canopy-scale estimates by dividing the daily integrated process rates by the daily leaf area and correcting for the within-canopy shading (Eqn 3). The lines (solid, ambient [CO_2]; dashed, elevated [CO_2]) were calculated by nonlinear least-squares regressions to Eqn 3 ($r^2 > 0.98$, $P < 0.0001$).

than under ambient [CO_2] (9.1 vs 12.7 nmol m^{-2} s^{-1}; Fig. 6). While the net assimilation fluxes were approximately of the same shape regardless of the growth environment, the isoprene emission fluxes showed distinct dynamic behaviors among the treatments. The maximum isoprene emission flux rates occur at different times, with the elevated [CO_2]-grown plants being about 2–3 d delayed, which is remarkable, as the inflection point t_i was only 1 d ahead.

Table 4 Temporal variation in hybrid aspen (*Populus tremula* × *Populus tremuloides*) leaf-scale net assimilation and isoprene emission fluxes according to the modified Chapman–Richards model (Eqn 3)

	$Q(t)$ (μmol m^{-2} s^{-1})	z_0 (μmol m^{-2} s^{-1})	$A_{max,leaf}$ (μmol m^{-2} s^{-1})	r (s^{-1})	c	t_i (d)	A_i (μmol m^{-2} s^{-1})	R_i (μmol m^{-2} s^{-2})
Net assimilation								
Ambient	4.44 ± 0.35	−0.10 ± 0.01	4.6 ± 0.6	0.193 ± 0.019	5.6 ± 2.0	9 ± 1	2.57 ± 0.13	0.86 ± 0.12
Elevated	3.83 ± 0.20	−0.27 ± 0.04*	6.1 ± 0.4*	0.211 ± 0.013*	6.9 ± 1.2	9 ± 1	3.62 ± 0.31*	1.23 ± 0.19*
P-value†	0.143	0.013	0.006	0.049	0.085	0.814	0.005	0.002
	$Q(t)$ (nmol m^{-2} s^{-1})	z_0 (nmol m^{-2} s^{-1})	$I_{max,leaf}$ (nmol m^{-2} s^{-1})	r (s^{-1})	c	t_i (d)	I_i (nmol m^{-2} s^{-1})	R_i (nmol m^{-2} s^{-2})
Isoprene emission								
Ambient	3.99 ± 0.22	0.000	10.5 ± 0.4	0.158 ± 0.009	7.6 ± 1.3	12 ± 1	5.43 ± 0.07	1.34 ± 0.14
Elevated	2.56 ± 0.36*	0.000	5.9 ± 1.0*	0.141 ± 0.009	5.3 ± 0.5*	11 ± 1	3.14 ± 0.42*	0.69 ± 0.17*
P-value†	0.009		0.001	0.138	0.005	0.449	0.003	0.037

$Q(t)$, the change in canopy light transmission; z_0, an offset parameter; $A_{max,leaf}$, the leaf scale maximum net assimilation flux; $I_{max,leaf}$, the maximum isoprene emission flux; parameters r and c are as defined in Table 2; the maximal process rate (R_i) is calculated at the inflection point (t_i, A_i or I_i) of the rising part of the curve.

*Significant difference between the mean values at $P < 0.05$.

†Differences in the mean values ($n = 10$ for all parameters) were compared between the treatments by paired t-tests after normality was confirmed.

Discussion

Effects of elevated [CO_2] on leaf traits and canopy development

Many previous studies that focused on plants grown under elevated [CO_2] have suggested that there is a link between 'up-regulation' and 'down-regulation' of leaf traits and carbon uptake (Luo *et al.*, 1998; Nowak *et al.*, 2004). In this study, the elevated [CO_2] treatment resulted in a higher maximum leaf area and leaf dry mass on the canopy scale as well as a higher leaf dry mass per unit area (Table 1), which is in line with previous findings on the effects of elevated [CO_2] (e.g. Sims *et al.*, 1998a,b; Miyazawa *et al.*, 2011). According to the fitted parameters characterizing canopy leaf area development, elevated [CO_2] stimulated the leaf area growth 1.4-fold. Together with doubled canopy-scale assimilation rate (Fig. 5) and the reported increase in leaf starch content (Sun *et al.*, 2012b), this suggests that elevated [CO_2] enhances the carbon supply for leaf construction, induces morphological changes and alters metabolic processes. In our study, plants grown under elevated [CO_2] had significantly higher foliage carbon content on a leaf area basis but not on a dry mass basis. Foliage nitrogen content showed the opposite response, with lower content on a dry mass basis but not on a leaf area basis. This is in agreement with earlier findings that, for a given nutrient supply, leaf N content per unit mass is reduced under elevated [CO_2] (Liu *et al.*, 2005).

Despite lower N content per unit dry mass, leaf area growth can still be enhanced under elevated [CO_2]. Taylor *et al.* (2008) observed a greater rate of canopy leaf area formation in establishing canopy, resulting in overall higher canopy LAI for the whole vegetation period. There is further evidence demonstrating that plants grown under elevated CO_2 maintain greater leaf area over time unless feedbacks as a result of soil nutrient limitations start to constrain foliage growth (Gielen *et al.*, 2003; Norby *et al.*, 2005; Luo *et al.*, 2006; Liberloo *et al.*, 2007). Our study further suggests that elevated [CO_2] is a key factor during canopy leaf area development. In particular, the maximum growth rate was doubled under elevated [CO_2] (Fig. 4, Table 2).

Increased daily net assimilation and isoprene emission rates on canopy level

Effects of growth [CO_2] on leaf-level net assimilation and isoprene emission rates have been reported in several studies (see the Introduction and Centritto *et al.*, 2004; Possell *et al.*, 2005; Wilkinson *et al.*, 2009; Possell & Hewitt, 2011; Sun *et al.*, 2012b). It has often been speculated that the down-regulation of isoprene emissions by elevated [CO_2] might be balanced by enhanced leaf area growth. The work of Possell *et al.* (2005, 2010), Possell & Hewitt (2011) and Centritto *et al.* (2004) suggested that, on a canopy or whole-plant level, no significant down-regulation in isoprene emission was seen, but in Rosenstiel *et al.* (2003), canopy-level isoprene emissions were also reduced under elevated [CO_2]. In our study, the daily isoprene emission rate was increased by a factor of 1.4 in plants grown under elevated [CO_2] as compared with plants grown in current ambient [CO_2]. Furthermore, the daily net assimilation rate was found to be doubled. Given the general enhancement of photosynthetic production and the overall moderate fraction of carbon going into isoprene synthesis (Sharkey & Yeh, 2001), differences in the supply of precursors for the isoprene synthesis seem to be unlikely across the studies. In fact, study-to-study differences in the degree of enhancement of foliage area expansion growth seem to provide an explanation for study-to-study differences in the patterns observed. Although leaf area increase moderated the leaf-level inhibition by elevated [CO_2] at the canopy scale, a much lower leaf area increase was observed in the study of Rosenstiel *et al.* (2003) than in our study.

The application of the growth model further emphasized that both canopy-scale net assimilation and isoprene emission rates followed leaf area developmental dynamics. This is in agreement with suggestions that isoprene emission could be controlled by the whole-plant carbon allocation pattern rather than by the availability of photosynthetically fixed carbon (Funk *et al.*, 1999). There is further evidence that part of the carbon emitted as isoprene comes from 'old' stored carbon (Kreuzwieser *et al.*, 2002; Trowbridge *et al.*, 2012), and thus, not only growth and photosynthesis, but also growth and isoprene emission can be partly regulated at the level of soluble carbon pools. In this context, it is relevant that elevated [CO_2] can shift the contributions of 'old' and recently fixed carbon to isoprene emission (Trowbridge *et al.*, 2012).

Downscaling to leaf-level processes

If the data are normalized per unit leaf area, the canopy processes can be scaled down to leaf level. As leaves are shaded by each other in a canopy and emission capacities acclimate to different environmental conditions inside the canopy, we emphasize that the canopy-scale rates calculated per unit leaf area are not directly comparable to single leaf measurements (Niinemets *et al.*, 2010a; Niinemets, 2012). Nevertheless, these 'leaf scale' estimates demonstrate the efficiency of unit leaf area for net assimilation and isoprene emission as determined by their prevailing environmental conditions in the canopy and metabolic capacity.

While the net assimilation flux at the leaf level was still enhanced by a factor of 1.4 under elevated [CO_2], the opposite was found for isoprene emission fluxes. Per unit leaf area, the maximum isoprene emission fluxes, adjusted to a changed light environment during leaf growth (Eqn 3), were *c.* 30% lower in plants grown under elevated [CO_2]. Given that total leaf area increased by a factor of 1.4, and the canopy isoprene emission rate was increased by the same factor, one might argue that there should be no effect if scaled to unit leaf area. However, such an argument is misleading, because of strongly nonlinear responses of isoprene emissions to light, implying that the contribution of upper canopy leaves is disproportionately larger than that of lower canopy leaves (Cescatti & Niinemets, 2004; Niinemets *et al.*, 2011; Niinemets, 2012). Furthermore, this 'canopy effect' changed with the expansion of plant canopy, underpinning the argument that canopy developmental state played an important role in affecting the average 'efficiency' of isoprene synthesis.

Furthermore, treatment-dependent changes in this dynamic behavior constitute an important result. In particular, earlier onset of reduction of the maximum flux in ambient [CO_2] leads to a less prominent difference between both treatments (Fig. 6). This difference might reflect earlier cessation of leaf growth and maturation under ambient [CO_2]. Thus, despite enhanced self-shading, the plants under elevated [CO_2] likely possessed a greater foliage area fraction that was still developing; the isoprene emission rate of these leaves was still increasing, while the emission capacity of all leaves in plants under ambient [CO_2] had already reached the maximum value.

The differences in net assimilation and isoprene emission in whole-canopy vs leaf-scale responses (enhancement of net assimilation at both canopy and leaf scales and enhancement of isoprene emission at canopy and reduction at leaf scale) and differences in temporal dynamics at the leaf scale emphasize the important difference in the control of photosynthesis and isoprene emission by light and CO_2 concentration. Light sensitivity, and thus responsiveness to within-canopy light gradients of net assimilation, decreases with increasing [CO_2] as a result of improved quantum yield, while the light sensitivity of isoprene emissions increases with increasing [CO_2] (Sun *et al.*, 2012b). Thus, complex interactions among canopy expansion, self-shading, leaf development and process dependence on light and [CO_2] collectively explain the differences in canopy- and leaf-level net assimilation and isoprene emissions.

What can we learn from the canopy-scale dynamics for large-scale isoprene emission modeling?

All recent approaches to model the impact of atmospheric [CO_2] on isoprene emissions are based on data on single leaf measurements expressed either per unit leaf area or per unit dry mass (Possell *et al.*, 2005; Wilkinson *et al.*, 2009; Possell & Hewitt, 2011). This is especially interesting as Possell *et al.* (2005, 2010) and also Pegoraro *et al.* (2005, 2006) were measuring whole-canopy gas exchange, but their model extensions of the Guenther *et al.* (1993, 1995) algorithm were based only on leaf area scaled measurements. Furthermore, extensive within-canopy variation in isoprene emission potentials (Harley *et al.*, 1996; Funk *et al.*, 2006; Niinemets *et al.*, 2010b) as well as greater light sensitivity of isoprene emission in plants grown under elevated [CO_2] (Sun *et al.*, 2012b) have not been considered. Use of a single leaf-scale emission estimate without considering modifications in canopy leaf area and within-canopy variation patterns, and altered light sensitivity, implies that single-leaf and whole-canopy growth [CO_2] responses will always be the same. As our results demonstrate, a leaf-scale reduction in isoprene emission does not necessarily correspond to canopy-scale reduction. The quality of the upscaling procedure from leaf to canopy, including the inherent within-canopy variation in emission capacity and light sensitivity, and, over the long term, consideration of leaf area dynamics, determines whether or not the canopy-scale modeled emissions will match the measurements. Therefore, a very first task should be to test whether the upscaled model estimations are in agreement with the measured values on the canopy scale.

Using a classic growth model (Eqns 1, 2 and S1–S8), we have shown that the developmental dynamic is an important factor to consider in fast-growing canopies. The key processes (leaf area growth, net assimilation, and isoprene emission rates) reached a stable state within 2–3 wk at the canopy level. This allows us to give robust estimates on the relative change of the process dynamics for plants grown under different [CO_2] treatments. As our approach excluded severe stresses, these estimates may correspond to an optimum situation and may need modification to account for stress events under natural field conditions. Nevertheless, isoprene emission is much less sensitive to stress than net

assimilation rate, and may even actually increase under mild drought and ozone stress (Loreto & Schnitzler, 2010; Niinemets, 2010a). At any rate, we suggest the use of information available on the canopy scale to constrain and verify model estimations that scale up from the leaf to canopy.

Scaling down from canopy to leaf allows for further insight into canopy-level modifications. Although one can simply calculate the emission rate per unit leaf area as the rate of canopy emission divided by canopy leaf area, the resulting variable carries no physiological meaning, because of within-canopy gradients in light and strongly nonlinear responses of isoprene emission to light. We note that in some early isoprene emission models, such an approach for scaling has been used (Pierce & Waldruff, 1991; Owen *et al.*, 2003), but as a result of integration errors, it is discouraged. To account for within-canopy changes, we included light gradient in the growth model that result in more or less correct inversion of the canopy-level emissions (Eqn 3, Fig. 6).

It has been suggested that, on the canopy scale, leaf-to-leaf differences are inherently integrated and, therefore, that scale may be more appropriate to model large-scale emission estimates as currently recommended by MEGAN (Guenther *et al.*, 2006). However, model parameters used in canopy-scale models also must come from canopy-scale measurements rather than from leaf-level estimates to avoid scaling errors (Niinemets *et al.*, 2010a for a discussion).

Conclusions

Our results show that, in hybrid aspen, the canopy-scale isoprene emission rate under elevated [CO_2] significantly exceeded that under ambient [CO_2]. The main reason for this difference was the stimulated growth of the canopy leaf area, which more than compensated for biochemical down-regulation of the isoprene synthesis pathway. We also showed that a portion of the inhibition of isoprene emission by elevated [CO_2] may be caused by the enhanced leaf area growth, if rescaled from canopy to leaf scale, resulting from acclimation to enhanced shading and lower within-canopy light intensities. However, greater responsiveness of isoprene emissions to light under elevated [CO_2], as demonstrated in a previous study (Sun *et al.*, 2012b), likely compensated for the reduced light intensity. Furthermore, different temporal dynamics of leaf-level estimates suggested different age structure of leaf populations among elevated and ambient [CO_2]. Thus, modeling canopy isoprene emissions without considering within-canopy patterns in leaf age, acclimation capacity and responsiveness to light is very problematic. In particular, use of 'average emission factors', one for elevated [CO_2] and one for ambient [CO_2], for upscaling to the canopy is not to be recommended, and might be very misleading.

Overall, we suggest that predictions of future isoprene emission responses to elevated [CO_2] should more explicitly consider canopy-scale processes. For regional- or global-scale estimations of isoprene emissions, we recommend the use of canopy-scale data, and encourage more experimental and modeling work to obtain these data. We also suggest that large-scale free air CO_2 enrichment (FACE) studies are needed to monitor long-term changes in canopy structure (LAI and biomass) in response to CO_2 enrichment and couple this to isoprene emission measurements.

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References

Affek HP, Yakir D. 2002. Protection by isoprene against singlet oxygen in leaves. *Plant Physiology* **129**: 269–277.

Affek HP, Yakir D. 2003. Natural abundance carbon isotope composition of isoprene reflects incomplete coupling between isoprene synthesis and photosynthetic carbon flow. *Plant Physiology* **131**: 1727–1736.

Arneth A, Niinemets Ü, Pressley S, Bäck J, Hari P, Karl T, Noe S, Prentice IC, Serça D, Hickler T *et al.* 2007. Process-based estimates of terrestrial ecosystem isoprene emissions: incorporating the effects of a direct CO_2-isoprene interaction. *Atmospheric Chemistry and Physics* **7**: 31–53.

Behnke K, Ehlting B, Teuber M, Bauerfeind M, Louis S, Hänsch R, Polle A, Bohlmann J, Schnitzler J-P. 2007. Transgenic, non-isoprene emitting poplars don't like it hot. *Plant Journal* **51**: 485–499.

von Bertalanffy L. 1957. Quantitative laws in metabolism and growth. *The Quarterly Review of Biology* **32**: 217–231.

Calfapietra C, Scarascia Mugnozza G, Karnosky DF, Loreto F, Sharkey TD. 2008. Isoprene emission rates under elevated CO_2 and O_3 in two field-grown aspen clones differing in their sensitivity to O_3. *New Phytologist* **179**: 55–61.

Causton DR, Venus JC. 1981. *The biometry of plant growth*. London, UK: Edward Arnold (Publishers) Ltd.

Centritto M, Nascetti P, Petrilli L, Raschi A, Loreto F. 2004. Profiles of isoprene emission and photosynthetic parameters in hybrid poplars exposed to free-Air CO_2 enrichment. *Plant, Cell & Environment* **27**: 403–412.

Cescatti A, Niinemets Ü. 2004. Sunlight capture. Leaf to landscape. In: Smith WK, Vogelmann TC, Chritchley C, eds. *Photosynthetic adaptation. Chloroplast to landscape*. Berlin, Germany: Springer Verlag, 42–85.

Cinege G, Louis S, Hänsch R, Schnitzler J-P. 2009. Regulation of isoprene synthase promoter by environmental and internal factors. *Plant Molecular Biology* **69**: 593–604.

Claeys M, Graham B, Vas G, Wang W, Vermeylen R, Pashynska V, Cafmeyer J, Guyon P, Andreae MO, Artaxo P *et al.* 2004. Formation of secondary organic aerosols through photooxidation of isoprene. *Science* **303**: 1173–1176.

Coleman JS, McConnaughay KDM, Bazzaz FA. 1993. Elevated CO_2 and plant nitrogen-use: is reduced tissue nitrogen concentration size-dependent? *Oecologia* **93**: 195–200.

Darbah JNT, Sharkey TD, Calfapietra C, Karnosky DF. 2010. Differential response of aspen and birch trees to heat stress under elevated carbon dioxide. *Environmental Pollution* **158**: 1008–1014.

Evans GC. 1972. *The quantitative analysis of plant growth*. Oxford, UK: Blackwell Science.

Fehsenfeld F, Calvert J, Fall R, Goldan P, Guenther AB, Hewitt CN, Lamb B, Liu S, Trainer M, Westberg H *et al.* 1992. Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry. *Global Biogeochemical Cycles* **6**: 389–430.

Fuentes J, Lerdau M, Atkinson R, Baldocchi D, Bottenheim J, Ciccioli P, Lamb B, Geron C, Gu L, Guenther A *et al.* 2000. Biogenic hydrocarbons in the

atmospheric boundary layer: a review. *Bulletin of the American Meteorological Society* **81**: 1537–1575.

Funk JL, Giardina CP, Knohl A, Lerdau MT. 2006. Influence of nutrient availability, stand age, and canopy structure on isoprene flux in a *Eucalyptus saligna* experimental forest. *Journal of Geophysical Research – Biogeosciences* **111**: G02012.

Funk JL, Jones CG, Lerdau MT. 1999. Defoliation effects on isoprene emission from *Populus deltoides*. *Oecologia* **118**: 333–339.

Gielen B, Liberloo M, Bogaert J, Calfapietra C, De Angelis P, Miglietta F, Scarascia-Mugnozza G, Ceulemans R. 2003. Three years of free-air CO_2 enrichment (POPFACE) only slightly affect profiles of light and leaf characteristics in closed canopies of *Populus*. *Global Change Biology* **9**: 1022–1037.

Guenther A, Hewitt CN, Erickson D, Fall R, Geron C, Graedel T, Harley P, Klinger L, Lerdau M, McKay WA *et al.* 1995. A global model of natural volatile organic compound emissions. *Journal of Geophysical Research* **100**: 8873–8892.

Guenther A, Karl T, Harley P, Wiedinmyer C, Palmer PI, Geron C. 2006. Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature). *Atmospheric Chemistry and Physics* **6**: 3181–3210.

Guenther AB, Zimmerman PR, Harley PC, Monson RK, Fall R. 1993. Isoprene and monoterpene emission rate variability: model evaluations and sensitivity analyses. *Journal of Geophysical Research* **98**: 12609–12617.

Häikiö E, Freiwald V, Julkunenen-Tiitto R, Beuker E, Holopainen T, Oksanen E. 2009. Differences in leaf characteristics between ozone-sensitive and ozone tolerant hybrid aspen (*Populus tremula* × *Populus tremuloides*) clones. *Tree Physiology* **29**: 53–66.

Hallquist M, Wenger JC, Baltensperger U, Rudich Y, Simpson D, Claeys M, Dommen J, Donahue NM, George C, Goldstein AH *et al.* 2009. The formation, properties and impact of secondary organic aerosol: current and emerging issues. *Atmospheric Chemistry and Physics* **9**: 5155–5236.

Harley P, Guenther A, Zimmerman P. 1996. Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (*Liquidambar styraciflua*) leaves. *Tree Physiology* **16**: 25–32.

Harley PC, Litvak ME, Sharkey TD, Monson RK. 1994. Isoprene emission from velvet bean leaves. Interactions among nitrogen availability, growth photon flux density, and leaf development. *Plant Physiology* **105**: 279–285.

Heald CL, Wilkinson MJ, Monson RK, Alo CA, Wang G, Guenther A. 2009. Response of isoprene emission to ambient CO_2 changes and implications for global budgets. *Global Change Biology* **15**: 1127–1140.

Hunt R. 1982. *Plant growth curves. The functional approach to plant growth analysis*. London, UK: Edward Arnold (Publishers) Ltd.

IPCC. 2007. *Working Group I Report: the physical basis of climate change*. URL http://ipcc-wg1.ucar.edu/wg1/wg1-report.html [accessed on 12 April 2012].

Kaplan JO, Folberth G, Hauglustaine DA. 2006. Role of methane and biogenic volatile organic compound sources in late glacial and Holocene fluctuations of atmospheric methane concentrations. *Global Biogeochemical Cycles* **20**: GB2016.

Kreuzwieser J, Graus M, Wisthaler A, Hansel A, Rennenberg H, Schnitzler J-P. 2002. Xylem-transported glucose as an additional carbon source for leaf isoprene formation in *Quercus robur*. *New Phytologist* **156**: 171–178.

Kroll JH, Ng NL, Murphy SM, Flagan RC, Seinfeld JH. 2005. Secondary organic aerosol formation from isoprene photooxidation under high-NOx conditions. *Geophysical Research Letters* **32**: L18808.

Kuhn U, Rottenberger S, Biesenthal T, Wolf A, Schebeske G, Ciccioli P, Brancaleoni E, Frattoni M, Tavares TM, Kesselmeier J. 2004. Seasonal differences in isoprene and light-dependent monoterpene emission by Amazonian tree species. *Global Change Biology* **10**: 663–682.

Kuzma J, Fall R. 1993. Leaf isoprene emission rate is dependent on leaf development and the level of isoprene synthase. *Plant Physiology* **101**: 435–440.

Li D, Chen Y, Shi Y, He X, Chen X. 2009. Impact of elevated CO_2 and O_3 concentrations on biogenic volatile organic compounds emissions from *Ginkgo biloba*. *Bulletin of Environmental Contamination and Toxicology* **82**: 473–477.

Li Z, Ratliff EA, Sharkey TD. 2011. Effect of temperature on postillumination isoprene emission in oak and poplar. *Plant Physiology* **155**: 1037–1046.

Liberloo M, Tulva I, Räim O, Kull O, Ceulemans R. 2007. Photosynthetic stimulation under long-term CO_2 enrichment and fertilization is sustained across a closed *Populus* canopy profile (EUROFACE). *New Phytologist* **173**: 537–549.

Lichtenthaler HK. 1999. The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**: 47–65.

Lichtenthaler HK, Schwender Jr, Disch A, Rohmer M. 1997. Biosynthesis of isoprenoids in higher plant chloroplasts proceeds via a mevalonate-independent pathway. *FEBS Letters* **400**: 271–274.

Liu L, King JS, Giardina CP. 2005. Effects of elevated concentrations of atmospheric CO_2 and tropospheric O_3 on leaf litter production and chemistry in trembling aspen and paper birch communities. *Tree Physiology* **25**: 1511–1522.

Liu ZG, Li FR. 2003. The generalized Chapman-Richards function and applications to tree and stand growth. *Journal of Forestry Research* **14**: 19–26.

Loivamäki M, Louis S, Cinege G, Zimmer I, Fischbach RJ, Schnitzler J-P. 2007. Circadian rhythms of isoprene biosynthesis in grey poplar leaves. *Plant Physiology* **143**: 540–551.

Loivamäki M, Mumm R, Dicke M, Schnitzler JP. 2008. Isoprene interferes with the attraction of bodyguards by herbaceous plants. *Proceedings of the National Academy of Sciences, USA* **105**: 17430–17435.

Long SP, Ainsworth EA, Rogers A, Ort DR. 2004. Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology* **55**: 591–628.

Loreto F, Centritto M, Barta C, Calfapietra C, Fares S, Monson RK. 2007. The relationship between isoprene emission rate and dark respiration rate in white poplar (*Populus alba* L.) leaves. *Plant, Cell & Environment* **30**: 662–669.

Loreto F, Schnitzler J-P. 2010. Abiotic stresses and induced BVOCs. *Trends in Plant Science* **15**: 154–166.

Loreto F, Sharkey T. 1993. On the relationship between isoprene emission and photosynthetic metabolites under different environmental conditions. *Planta* **189**: 420–424.

Loreto F, Velikova V. 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology* **127**: 1781–1787.

Luo Y, Hui D, Zhang D. 2006. Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. *Ecology* **87**: 53–63.

Luo Y, Sims DA, Griffin KL. 1998. Nonlinearity of photosynthetic responses to growth in rising atmospheric CO_2: an experimental and modeling study. *Global Change Biology* **4**: 173–183.

Magel E, Mayrhofer S, Müller A, Zimmer I, Hampp R, Schnitzler JP. 2006. Photosynthesis and substrate supply for isoprene biosynthesis in poplar leaves. *Atmospheric Environment* **40**(Suppl 1): 138–151.

Mayrhofer S, Teuber M, Zimmer I, Louis S, Fischbach RJ, Schnitzler J-P. 2005. Diurnal and seasonal variation of isoprene biosynthesis-related genes in grey poplar leaves. *Plant Physiology* **139**: 474–484.

McConnaughay KDM, Coleman JS. 1999. Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. *Ecology* **80**: 2581–2593.

Miyazawa S-I, Warren CR, Turpin DH, Livingston NJ. 2011. Determination of the site of CO_2 sensing in poplar: is the area-based N content and anatomy of new leaves determined by their immediate CO_2 environment or by the CO_2 environment of mature leaves? *Journal of Experimental Botany* **62**: 2787–2796.

Monson RK, Harley PC, Litvak ME, Wildermuth M, Guenther AB, Zimmerman PR, Fall R. 1994. Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves. *Oecologia* **99**: 260–270.

Monson RK, Hills AJ, Zimmerman PR, Fall RR. 1991. Studies of the relationship between isoprene emission rate and CO_2 or photon-flux density using a real-time isoprene analyzer. *Plant, Cell & Environment* **14**: 517–523.

Monson RK, Trahan N, Rosenstiel TN, Veres P, Moore D, Wilkinson M, Norby RJ, Volder A, Tjoelker MG, Briske DD *et al.* 2007. Isoprene emission from terrestrial ecosystems in response to global change: minding the gap between models and observations. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **365**: 1677–1695.

Niinemets Ü. 2010a. Mild versus severe stress and BVOCs: thresholds, priming and consequences. *Trends in Plant Science* **15**: 145–153.

Niinemets Ü. 2010b. Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. *Forest Ecology and Management* **260**: 1623–1639.

Niinemets Ü. 2012. Whole plant photosynthesis. In: Flexas J, Loreto F, Medrano H, eds. *Terrestrial photosynthesis in a changing environment. The molecular, physiological and ecological bases of photosynthesis driving its response to the environmental changes.* Cambridge, UK: Cambridge University Press, 403–428.

Niinemets Ü, Copolovici L, Hüve K. 2010b. High within-canopy variation in isoprene emission potentials in temperate trees: implications for predicting canopy-scale isoprene fluxes. *Journal of Geophysical Research – Biogeosciences* **115**: G04029.

Niinemets Ü, Kuhn U, Harley PC, Staudt M, Arneth A, Cescatti A, Ciccioli P, Copolovici L, Geron C, Guenther AB *et al.* 2011. Estimation of isoprenoid emission factors from enclosure studies: measurements, data processing, quality and standardized measurement protocols. *Biogeosciences* **8**: 2209–2246.

Niinemets Ü, Monson RK, Arneth A, Ciccioli P, Kesselmeier J, Kuhn U, Noe SM, Peñuelas J, Staudt M. 2010a. The leaf-level emission factor of volatile isoprenoids: caveats, model algorithms, response shapes and scaling. *Biogeosciences* **7**: 1809–1832.

Niinemets Ü, Tenhunen JD, Harley PC, Steinbrecher R. 1999. A model of isoprene emission based on energetic requirements for isoprene synthesis and leaf photosynthetic properties for *Liquidambar* and *Quercus*. *Plant, Cell & Environment* **22**: 1319–1335.

Norby RJ, DeLucia EH, Gielen B, Calfapietra C, Giardina CP, King JS, Ledford J, McCarthy HR, Moore DJP, Ceulemans R *et al.* 2005. Forest response to elevated CO_2 is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences, USA* **102**: 18052–18056.

Nowak RS, Ellsworth DS, Smith SD. 2004. Functional responses of plants to elevated atmospheric CO_2– do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist* **162**: 253–280.

Oksanen E, Sober J, Karnosky DF. 2001. Impacts of elevated CO_2 and/or O_3 on leaf ultrastructure of aspen (*Populus tremuloides*) and birch (*Betula papyrifera*) in the Aspen FACE experiment. *Environmental Pollution* **115**: 437–446.

Owen SM, MacKenzie AR, Stewart H, Donovan R, Hewitt CN. 2003. Biogenic volatile organic compound (VOC) emission estimates from an urban tree canopy. *Ecological Applications* **13**: 927–938.

Owen SM, Peñuelas J. 2005. Opportunistic emissions of volatile isoprenoids. *Trends in Plant Science* **10**: 420–426.

Pegoraro E, Abrell L, Van Haren J, Barron-Gafford G, Grieve KA, Malhi Y, Murthy R, Lin G. 2005. The effect of elevated atmospheric CO_2 and drought on sources and sinks of isoprene in a temperate and tropical rainforest mesocosm. *Global Change Biology* **11**: 1234–1246.

Pegoraro E, Rey ANA, Abrell L, Van Haren J, Lin G. 2006. Drought effect on isoprene production and consumption in Biosphere 2 tropical rainforest. *Global Change Biology* **12**: 456–469.

Peñuelas J, Staudt M. 2010. BVOCs and global change. *Trends in Plant Science* **15**: 133–144.

Pienaar LV, Turnbull KJ. 1973. The Chapman-Richards generalization of Von Bertalanffy's growth model for basal area growth and yield in even-aged stands. *Forest Science* **19**: 2–22.

Pierce TE, Waldruff PS. 1991. PC-BEIS – a personal-computer version of the biogenic emissions inventory system. *Journal of the Air & Waste Management Association* **41**: 937–941.

Possell M, Hewitt CN. 2011. Isoprene emissions from plants are mediated by atmospheric CO_2 concentrations. *Global Change Biology* **17**: 1595–1610.

Possell M, Nicholas Hewitt C, Beerling DJ. 2005. The effects of glacial atmospheric CO_2 concentrations and climate on isoprene emissions by vascular plants. *Global Change Biology* **11**: 60–69.

Possell M, Ryan A, Vickers C, Mullineaux P, Hewitt C. 2010. Effects of fosmidomycin on plant photosynthesis as measured by gas exchange and chlorophyll fluorescence. *Photosynthesis Research* **104**: 49–59.

Rapparini F, Baraldi R, Miglietta F, Loreto F. 2004. Isoprenoid emission in trees of *Quercus pubescens* and *Quercus ilex* with lifetime exposure to naturally high CO_2 environment. *Plant, Cell & Environment* **27**: 381–391.

Rasulov B, Copolovici L, Laisk A, Niinemets Ü. 2009. Postillumination isoprene emission: *in vivo* measurements of dimethylallyldiphosphate pool size and isoprene synthase kinetics in aspen leaves. *Plant Physiology* **149**: 1609–1618.

Rasulov B, Hüve K, Bichele I, Laisk A, Niinemets Ü. 2010. Temperature response of isoprene emission *in vivo* reflects a combined effect of substrate limitations and isoprene synthase activity: a kinetic analysis. *Plant Physiology* **154**: 1558–1570.

Rosenstiel TN, Potosnak MJ, Griffin KL, Fall R, Monson RK. 2003. Increased CO_2 uncouples growth from isoprene emission in an agriforest ecosystem. *Nature* **421**: 256–259.

Sanadze G. 2010. Photobiosynthesis of isoprene as an example of leaf excretory function in the light of contemporary thermodynamics. *Russian Journal of Plant Physiology* **57**: 1–6.

Sasaki K, Ohara K, Yazaki K. 2005. Gene expression and characterization of isoprene synthase from *Populus alba*. *FEBS Letters* **579**: 2514–2518.

Schnitzler J-P, Graus M, Kreuzwieser J, Heizmann U, Rennenberg H, Wisthaler A, Hansel A. 2004. Contribution of different carbon sources to isoprene biosynthesis in poplar leaves. *Plant Physiology* **135**: 152–160.

Scholefield PA, Doick KJ, Herbert BMJ, Hewitt CNS, Schnitzler JP, Pinelli P, Loreto F. 2004. Impact of rising CO_2 on emissions of volatile organic compounds: isoprene emission from *Phragmites australis* growing at elevated CO_2 in a natural carbon dioxide spring. *Plant, Cell & Environment* **27**: 393–401.

Sharkey TD, Loreto F, Delwiche CF. 1991. High carbon dioxide and sun/shade effects on isoprene emission from oak and aspen tree leaves. *Plant, Cell & Environment* **14**: 333–338.

Sharkey TD, Singsaas EL. 1995. Why plants emit isoprene. *Nature* **374**: 769.

Sharkey TD, Wiberley AE, Donohue AR. 2008. Isoprene emission from plants: why and how. *Annals of Botany* **101**: 5–18.

Sharkey TD, Yeh S. 2001. Isoprene emissions from plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**: 407–436.

Sims DA, Seemann JR, Luo Y. 1998a. Elevated CO_2 concentration has independent effects on expansion rates and thickness of soybean leaves across light and nitrogen gradients. *Journal of Experimental Botany* **49**: 583–591.

Sims DA, Seemann JR, Luo Y. 1998b. The significance of differences in the mechanisms of photosynthetic acclimation to light, nitrogen and CO_2 for return on investment in leaves. *Functional Ecology* **12**: 185–194.

Singsaas EL, Sharkey TD. 1998. The regulation of isoprene emission responses to rapid leaf temperature fluctuations. *Plant, Cell & Environment* **21**: 1181–1188.

Singsaas EL, Sharkey TD. 2000. The effects of high temperature on isoprene synthesis in oak leaves. *Plant, Cell & Environment* **23**: 751–757.

Sun Z, Copolovici L, Niinemets Ü. 2012a. Can the capacity for isoprene emission acclimate to environmental modifications during autumn senescence in temperate deciduous tree species *Populus tremula*? *Journal of Plant Research* **125**: 263–274.

Sun Z, Niinemets Ü, Hüve K, Noe SM, Rasulov B, Copolovici L, Vislap V. 2012b. Enhanced isoprene emission capacity and altered light responsiveness in aspen grown under elevated atmospheric CO_2 concentration. *Global Change Biology* **18**: 3423–3440.

Taylor G, Tallis MJ, Giardina CP, Percy KE, Miglietta F, Gupta PS, Gioli B, Calfapietra C, Gielen B, Kubiske ME *et al.* 2008. Future atmospheric CO_2 leads to delayed autumnal senescence. *Global Change Biology* **14**: 264–275.

Tognetti R, Johnson JD, Michelozzi M, Raschi A. 1998. Response of foliar metabolism in mature trees of *Quercus pubescens* and *Quercus ilex* to long-term elevated CO_2. *Environmental and Experimental Botany* **39**: 233–245.

Trowbridge AM, Asensio D, Eller ASD, Way DA, Wilkinson MJ, Schnitzler J-P, Jackson RB, Monson RK. 2012. Contribution of various carbon sources toward isoprene biosynthesis in poplar leaves mediated by altered atmospheric CO_2 concentrations. *PLoS ONE* **7**: e32387.

Velikova V, Tsonev T, Barta C, Centritto M, Koleva D, Stefanova M, Busheva M, Loreto F. 2009. BVOC emissions, photosynthetic characteristics and changes in chloroplast ultrastructure of *Platanus orientalis* L. exposed to elevated CO_2 and high temperature. *Environmental Pollution* **157**: 2629–2637.

Velikova V, Várkonyi Z, Szabó M, Maslenkova L, Nogues I, Kovács L, Peeva V, Busheva M, Garab G, Sharkey TD *et al.* 2011. Increased thermostability of thylakoid membranes in isoprene-emitting leaves probed with three biophysical techniques. *Plant Physiology* **157**: 905–916.

Vickers CE, Gershenzon J, Lerdau MT, Loreto F. 2009. A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nature Chemical Biology* **5**: 283–291.

Wiberley AE, Linskey AR, Falbel TG, Sharkey TD. 2005. Development of the capacity for isoprene emission in kudzu. *Plant, Cell & Environment* **28**: 898–905.

Wiedinmyer C, Tie X, Guenther A, Neilson R, Granier C. 2006. Future changes in biogenic isoprene emissions: how might they affect regional and global atmospheric chemistry? *Earth Interactions* **10**: 1–19.

Wilkinson MJ, Monson RK, Trahan N, Lee S, Brown E, Jackson RB, Polley HW, Fay PA, Fall RAY. 2009. Leaf isoprene emission rate as a function of atmospheric CO_2 concentration. *Global Change Biology* **15**: 1189–1200.

Williams J, Roberts JM, Fehsenfeld FC, Bertman SB, Buhr MP, Goldan PD, Hübler G, Kuster WC, Ryerson TB, Trainer M *et al.* 1997. Regional ozone from biogenic hydrocarbons deduced from airborne measurements of PAN, PPN, and MPAN. *Geophysical Research Letters* **24**: 1099–1102.

Yin X, Goudriaan JAN, Lantinga EA, Vos JAN, Spiertz HJ. 2003. A flexible sigmoid function of determinate growth. *Annals of Botany* **91**: 361–371.

Zimmer W, Brüggemann N, Emeis S, Giersch C, Lehning A, Steinbrecher R, Schnitzler JP. 2000. Process-based modeling of isoprene emission by oak leaves. *Plant, Cell & Environment* **23**: 585–595.

Supporting Information

Additional supporting information may be found in the online version of this article.

Methods S1 Eqns S1–S4, the detailed form of the integrated Eqn 1 and parameter transformations to reach to Eqn 2; Eqns S5–S8, the detailed solution of the second derivative of Eqn 2 and calculations of key parameters of fastest growth rate and time of fastest growth; Eqns S9–S12, details to connect the Lambert–Beer law to Eqn 2.

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Enhanced isoprene emission capacity and altered light responsiveness in aspen grown under elevated atmospheric CO_2 concentration

ZHIHONG SUN*, ÜLO NIINEMETS*, KATJA HÜVE*, STEFFEN M. NOE*, BAHTIJOR RASULOV†, LUCIAN COPOLOVICI* and VIVIAN VISLAP*

*Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51014, Estonia, †Institute of Molecular and Cell Biology, University of Tartu, Riia 23, Tartu 51010, Estonia

Abstract

Controversial evidence of CO_2-responsiveness of isoprene emission has been reported in the literature with the response ranging from inhibition to enhancement, but the reasons for such differences are not understood. We studied isoprene emission characteristics of hybrid aspen (*Populus tremula* x *P. tremuloides*) grown under ambient (380 μmol mol^{-1}) and elevated (780 μmol mol^{-1}) [CO_2] to test the hypothesis that growth [CO_2] effects on isoprene emission are driven by modifications in substrate pool size, reflecting altered light use efficiency for isoprene synthesis. A novel *in vivo* method for estimation of the pool size of the immediate isoprene precursor, dimethylallyldiphosphate (DMADP) and the activity of isoprene synthase was used. Growth at elevated [CO_2] resulted in greater leaf thickness, more advanced development of mesophyll and moderately increased photosynthetic capacity due to morphological "upregulation", but isoprene emission rate under growth light and temperature was not significantly different among ambient- and elevated-[CO_2]-grown plants independent of whether measured at 380 μmol mol^{-1} or 780 μmol mol^{-1} CO_2. However, DMADP pool size was significantly less in elevated-[CO_2]-grown plants, but this was compensated by increased isoprene synthase activity. Analysis of CO_2 and light response curves of isoprene emission demonstrated that the [CO_2] for maximum isoprene emission was shifted to lower [CO_2] in elevated-[CO_2]-grown plants. The light-saturated isoprene emission rate ($I_{max,Q}$) was greater, but the quantum efficiency at given $I_{max,Q}$ was less in elevated-[CO_2]-grown plants, especially at higher CO_2 measurement concentration, reflecting stronger DMADP limitation at lower light and higher [CO_2]. These results collectively demonstrate important shifts in light and CO_2-responsiveness of isoprene emission in elevated-[CO_2]-acclimated plants that need consideration in modeling isoprene emissions in future climates.

Keywords: emission modeling, isoprene emission, isoprenoid precursors, light response, physiological adaptation, quantum yield, structural adaptation

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Introduction

Isoprene is a dominant volatile hydrocarbon emitted from vegetation, especially by many tree species (Fehsenfeld *et al*., 1992; Guenther *et al*., 2006; Sharkey *et al*., 2008) with important protective functions in plant heat and oxidative stress resistance (e.g. Sharkey & Singsaas, 1995; Loreto *et al*., 2001b; Behnke *et al*., 2007). As a highly reactive molecule, isoprene also plays a major role in tropospheric chemistry and climate change, in particular in ozone and secondary organic aerosol formation (e.g. Fehsenfeld *et al*., 1992; Claeys *et al*., 2004; Kroll *et al*., 2006). Given the importance of isoprene, there is major interest in quantitative prediction of plant isoprene emissions both under current and future climates (Guenther *et al*., 2006; Monson *et al*., 2007; Grote & Niinemets, 2008).

Isoprene is formed in chloroplasts by isoprene synthase from its immediate precursor dimethylallyldiphosphate (DMADP) (Sharkey *et al*., 2008). DMADP synthesis according to 1-deoxy-D-xylulose-phosphate/2-C-methylerythritol 5-phosphate (DOXP/MEP) starts by condensation of pyruvate (Pyr) and the primary photosynthetic product glyceraldehyde-3-phosphate (GAP) (Lichtenthaler, 1999). Thus, isoprene emission is tightly bound to leaf photosynthetic carbon metabolism (Loreto & Sharkey, 1993; Ghirardo *et al*., 2011; Trowbridge *et al*., 2012). In fact, hyperbolic dependence of isoprene emissions on light and an Arrhenius-type temperature response with an optimum are qualitatively similar to dependencies of photosynthesis, and especially photosynthetic electron transport on these environmental drivers (Monson & Fall, 1989; Loreto & Sharkey, 1990; Harley *et al*., 1996; Niinemets *et al*.,

Correspondence: Ülo Niinemets, tel. + 37 253 457 189, fax + 37 273 137 38, e-mail: ylo.niinemets@emu.ee

1999). Available empirical and semi-mechanistic models based on the light and temperature responses analogous to photosynthesis describe isoprene emissions under current ambient CO_2 concentrations ($[CO_2]$) relatively well for non-stressed plants (Guenther *et al*., 1993; Zimmer *et al*., 2000; Arneth *et al*., 2007; Niinemets *et al*., 2010; Monson *et al*., 2012).

Differently from current conditions, modeling isoprene emissions to future climates is associated with major uncertainties. The instantaneous dependencies of isoprene emissions and photosynthesis on instantaneous $[CO_2]$ are very different. Photosynthesis typically increases monotonically with increasing $[CO_2]$ until saturation, whereas the CO_2 dependence of isoprene emission is characterized by an asymmetric curve with an optimum at low $[CO_2]$, below which isoprene emission decreases rapidly and above which isoprene emissions decrease relatively slowly (Loreto & Sharkey, 1990; Rasulov *et al*., 2009b). The mechanisms of instantaneous CO_2-responses of isoprene emission are currently not fully understood, but it has been demonstrated that changes in isoprene emissions are paralleled by changes in DMADP pool size over the entire CO_2 response curve, suggesting that the CO_2-response of isoprene emission reflects substrate-dependent limitation (Rosenstiel *et al*., 2003; Rasulov *et al*., 2009b; Possell & Hewitt, 2011). Several hypotheses have been offered to explain CO_2-dependent DMADP pool size variation, including limitation of DMADP formation by leaf ATP level (Rasulov *et al*., 2009b) and limitation by cytosolic processes (Rosenstiel *et al*., 2003; Monson *et al*., 2009; Wilkinson *et al*., 2009).

Assuming that Pyr needed for DMADP formation in chloroplasts comes from cytosolic phosphoenolpyruvate (PEP), and postulating that cytosolic PEP level is driven by CO_2-mediated increases in PEP carboxylase activity, instantaneous CO_2 responses were recently empirically modeled assuming a competition for PEP by cytosolic processes and chloroplast isoprene synthesis (Wilkinson *et al*., 2009; Possell & Hewitt, 2011). This model has further been applied to explain future isoprene emissions under globally rising CO_2 concentrations (Heald *et al*., 2009; Possell & Hewitt, 2011).

The key assumption in such an approach is that isoprene emission response to instantaneous increase in $[CO_2]$ and to long-term $[CO_2]$ elevation can be explained by the same mechanism, making it possible to simply extrapolate from instantaneous $[CO_2]$ responses of isoprene emissions to future emissions. Yet, long-term $[CO_2]$ elevation can affect isoprene emissions not only because of changes in DMADP pool size but also due to changes in isoprene synthase activity, i.e., leading to adaptive changes. Currently, adaptive changes driven by growth $[CO_2]$ are considered as a key gap in understanding biochemistry of isoprene formation (Sharkey, 2009), but identification of possible adaptive changes in literature is difficult as isoprene emission measurements have often only been carried out under growth conditions, i.e., ambient-$[CO_2]$-grown plants measured under ambient $[CO_2]$ and elevated-$[CO_2]$-grown plants measured under elevated $[CO_2]$ (Rosenstiel *et al*., 2003; Possell *et al*., 2004), confounding instantaneous CO_2 effects and possible adaptive modifications.

Furthermore, available data on modifications of isoprene emission capacity by growth $[CO_2]$ when measured under common CO_2 concentration are contrasting. Some studies demonstrate reduced emission capacity in elevated-CO_2-grown plants, including *Acacia nigrescens* (Possell & Hewitt, 2011), *Liquidambar styraciflua* (Monson *et al*., 2007; Wilkinson *et al*., 2009), *Populus tremuloides* (Darbah *et al*., 2010 one clone), (Sharkey *et al*., 1991), *Eucalyptus globulus*, *P. tremuloides* and *P. deltoides* (Wilkinson *et al*., 2009), *Phragmites australis* (Scholefield *et al*., 2004), and *Platanus orientalis* (Velikova *et al*., 2009). However, other studies have either found no significant change, including *P. tremuloides* (Darbah *et al*., 2010 another aspen clone), (Calfapietra *et al*., 2007, 2008), *P. x canescens* (Trowbridge *et al*., 2012), *Populus alba* (Loreto *et al*., 2001a, 2007; Brilli *et al*., 2007b), *Quercus chapmanii* (Buckley, 2001) and *Quercus pubescens* (Rapparini *et al*., 2004), or even increased emissions in *Gingko biloba* (Li *et al*., 2009), *Q. pubescens* (Tognetti *et al*., 1998) and *Q. rubra* (Sharkey *et al*., 1991). This large study-to-study variability is currently not understood, but suggests that acclimation of isoprene emission capacity to elevated $[CO_2]$ depends both on changes in isoprene synthase activity and alterations in DMADP pool size that may both increase or decrease under elevated $[CO_2]$ or the integrated response may be dominated by one of them. To our knowledge, both these factors have been only analyzed simultaneously in the study of Possell & Hewitt (2011), where reduced emission capacity was associated with both decreased isoprene synthase activity and DMADP pool size.

At any rate, these controversies among the studies suggest that the influence of growth $[CO_2]$ on isoprene emissions cannot be explained by the same mechanism as instantaneous $[CO_2]$ responses. Another potential shortcoming of current emission models including CO_2-control is that the CO_2 effects are considered independent of other environmental drivers, light, and temperature (Wilkinson *et al*., 2009; Possell & Hewitt, 2011). As both instantaneous (Rasulov *et al*., 2009b) and growth $[CO_2]$ (Possell & Hewitt, 2011) can alter DMADP pool size in addition to light and temperature-driven modifications in DMADP pool size (Rosenstiel

et al., 2002; Rasulov *et al*., 2009b, 2010; Li *et al*., 2011), [CO_2] effects can potentially alter isoprene emission responses to other environmental drivers too. Such potential changes in light and temperature responsiveness of isoprene emissions in plants grown under different [CO_2] have not yet been studied to our knowledge.

We have recently developed an *in vivo* method to separate the controls on isoprene emission due to isoprene synthase activity and DMADP pool size (Rasulov *et al*., 2009a,b, 2011). Here we employ this method to gain insight into growth-[CO_2]-dependent alterations in the share of emission controls by DMADP pool size and isoprene synthase activity. We test the hypotheses (1) that growth [CO_2] responsiveness of isoprene emission is determined by modifications in isoprene synthase activity, and (2) that alteration in the share of emission limitation by synthase activity and DMADP pool size modifies the emission responsiveness to instantaneous changes in key environmental drivers. Strong interactive effect between growth [CO_2] and instantaneous [CO_2] and light responses observed in our study highlights a major gap in all available models predicting future emissions.

Materials and methods

Plant material and growth system

Two-year-old saplings of hybrid aspen (*Populus tremuloides* Michx. x *P. tremula* L.) clone H200 were selected for the experiments (for the clone Rasulov *et al*., 2009b, 2010). The potted ca. 0.2 m tall plants were kept in a cold room at −2 °C in dormant state, and for each experimental run, a plant lot for the experiments was moved to 20 °C 4 days prior to the start of the treatment to break the dormancy.

After dormancy was broken, saplings with swelling buds were installed in a four-chamber open gas-exchange system specially designed for plant growth and for long-term continuous measurements of plant gas-exchange activities (Fig. 1). The chambers of 12.5 L volume (diameter 0.2 m, height 0.4 m) were made of glass and accommodated an individual sapling in each case. The chamber bottom was made of two glass plates with openings for fan, temperature sensor, and gas input and exhaust ports and for plant stem between the two plates (Rasulov *et al*., 2009a for the system principle). All tubing and connections were made of Teflon and stainless steel. The seal was close to ideal between the glass chamber and bottom plates, and modeling putty was only needed to seal off the site of plant enclosure. The chambers operated under slight overpressure of a few mbar to avoid uncontrolled leakage of air. Flow rate through each individual chamber was controlled at 7.5 L min^{-1} by calibrated capillaries (Laisk & Oja, 1998 for details). This flow rate, and resulting chamber response time are at the higher end of similar-sized whole plant chambers (Niinemets, 2012 for a review of the performance of different-sized whole plant chambers). CO_2 could be added to the system through a capillary and the concentration in each individual chamber controlled by manostatic mixers (Laisk & Oja, 1998). Whenever needed, CO_2 concentration could also be reduced below the ambient by a KOH scrubber (Fig. 1). For gas-exchange measurements, the chamber ports were sequentially switched between reference and measurement modes (Fig. 1). Each individual chamber was lit by four 50 W halogen lamps providing between 500 μmol m^{-2} s^{-1} (start of the experiment) and 800 μmol m^{-2} s^{-1} (end of the experiment) light at the top of the plants. In these experiments, the photoperiod length was 12 h, and thus, the daily integrated light level corresponds to ca. 60% of daily seasonal average integrated quantum flux density in temperate ecosystems (e.g. Niinemets, 2010).

The chamber air temperature was measured using thermistors (NTC thermistor, model CCA-001, RTI Electronics, Inc., St. Anaheim, CA, USA) and day/night air temperatures were maintained at 28–30/23 °C. Relative humidity air was kept at 60%. As upon placement into the chambers, the bud-burst had not yet occurred, whole leaf development was completed inside the chambers under the set [CO_2] conditions. Chambers 1 and 3 (Fig. 1) were kept at ambient CO_2 concentration (average ± SD) of 380 ± 10 μmol mol^{-1}, whereas chambers 2 and 4 were treated with elevated CO_2 concentration of 780 ± 10 μmol mol^{-1}.

The plants were grown in 3 L plastic pots filled with sand and peat mixture (1 : 1), and watered daily to field capacity with tap water. To maintain optimum nutrient supply, every fifth day, each plant was fertilized with NPK complex fertilizer solution (3.5 : 2.3 : 5.0 of N : P_2O_5 : K_2O with minor elements Fe, Mn, and Zn; Vito, Podriba, Latvia) diluted in 300 mL tap water such that each plant received 0.104 g N, 0.030 g P and 0.123 g K on each fertilization occasion. The leaf-level measurements were started when plants were just about to reach the top of the chamber, 30–40 days after the start of the treatment. The experiment was replicated four times, altogether with 16 plants in two treatment CO_2 concentrations.

Leaf-level net assimilation and isoprene emission measurements

We used a Walz GFS-3000 gas-exchange fluorescence system equipped with a LED-Array/PAM-Fluorometer 3055-FL (Walz GmbH, Effeltrich, Germany) together with the Fast isoprene sensor (FIS, Hills-Scientific, Boulder, CO, USA) for combined measurements of photosynthetic characteristics and isoprene emission rates. The isoprene sensor was integrated in the gas-exchange system through a bypass loop with three way valves, and operated as detailed previously (Rasulov *et al*., 2009a). The FIS analyzer was calibrated frequently with standard gas containing 4.47 ppm isoprene in N_2 (Hills-Scientific, Boulder, CO, USA).

For the measurements, the plant was taken out from the chamber, the measurement leaf was enclosed in the gas-exchange system, and baseline conditions of 500 μmol m^{-2} s^{-1} light, leaf temperature of 30 °C, and relative humidity of 60%

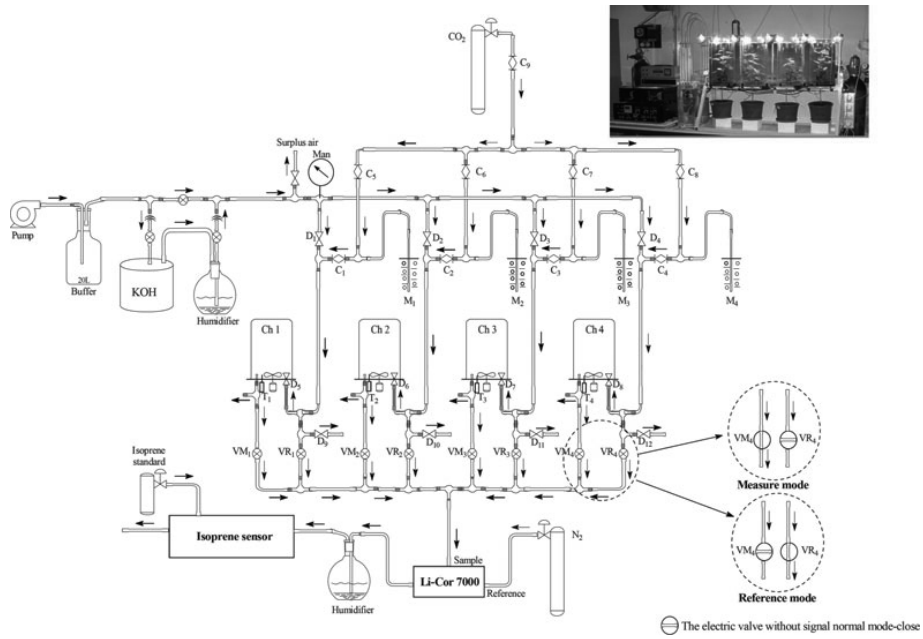

Fig. 1 Flow scheme and photograph of the computer-controlled four-chamber (Ch 1–Ch 4) open gas-exchange system for plant growth and continuous measurements of whole plant gas-exchange and isoprene emission rate. The flow in the system was controlled by calibrated diaphragms D_1–D_{12} for regulation of large flows and capillaries C_1–C_8 for fine-tuning. CO_2 concentration could be controlled by a KOH scrubber for below-ambient concentrations, whereas for higher concentrations, CO_2 could be added by a capillary (C_9) and the CO_2 concentration in individual chamber was controlled by manostatic gas mixers M_1–M_4 (Laisk & Oja, 1998 for details). Electronic valves VM1-VM4 and VR1-VR4 were used to switch the chamber in and output ports sequentially between measurement and reference modes. The chambers of 12.5 L were made of glass and illuminated individually by four 50 W halogen lamps. Chamber bottom was made of two glass plates that had openings for fan, temperature sensor (T_1–T_4), and gas input and output ports and for enclosure of the plant between the plates. All tubing and fittings were made of polytetrafluoroethylene (Teflon) and stainless steel.

were established. The baseline chamber CO_2 concentration was 380 μmol mol^{-1} for ambient-[CO_2]-grown and 780 μmol mol^{-1} for elevated-[CO_2]-grown plants. The leaf was maintained at these conditions until stomata fully opened and leaf net assimilation and transpiration rates and isoprene emission rates stabilized, typically 20–30 min after enclosure of the leaf in the cuvette. After stabilization, net assimilation rate (A) and isoprene emission (I) rate under these conditions were recorded. Thereafter, chamber [CO_2] was raised to 780 μmol mol^{-1} (ambient-[CO_2]-grown plants) or reduced to 380 μmol mol^{-1} (elevated-[CO_2]-grown plants), and the leaf was stabilized until the steady-state gas-exchange rates were achieved, and net assimilation and isoprene emission rates were recorded again at the other chamber [CO_2], and the leaf was returned to corresponding baseline [CO_2]. Under these conditions, the leaf was stabilized until the initial gas-exchange rates were reached again. Separate experiments demonstrated that the measurement sequence (either higher [CO_2] or lower [CO_2] first) did not alter the rates achieved in either of the treatments, but we considered the start of the measurements at growth [CO_2] more appropriate.

In vivo *measurements of the dimethylallyldiphosphate (DMADP) pool size and the rate constant of isoprene synthase (IspS)*

We employed the method of rapid light-dark transients of Rasulov *et al*. (2009a, 2010) for estimations of *in vivo* DMADP pool size and isoprene synthase rate constant. Estimation of DMADP pool size relies on the assumption that the initial post-tillumination burst of isoprene emission for 150–200 s after switching off the light (Supporting Information, Fig. S1) primarily originates from DMADP synthesized in light before the darkening with a small contribution of isopentenyldiphosphate pool (Rasulov *et al*., 2010, 2011; Li *et al*., 2011). Thus, DMADP pool size is equal to the integral of isoprene emission

rate after switching off the light (Rasulov *et al*., 2010, 2011; Li *et al*., 2011).

As the isoprene synthase operates at increasingly lower concentrations of DMADP after leaf darkening, paired values of isoprene emission rate $I(t)$ vs. remaining DMADP pool size at time t can also be derived from the light/dark transients of isoprene emission. These paired values of $I(t)$ vs. DMADP pool size over the entire darkening transient constitute the kinetic response curve of isoprene synthase, and the initial slope of this relationship provides the rate constant of isoprene synthase (K, s^{-1}) (Rasulov *et al*., 2010, 2011; Li *et al*., 2011).

In this study, DMADP pool size and K were estimated after the steady-state values of I_{380} and I_{780} had been recorded and the leaf had been stabilized again at corresponding [CO_2]. After stabilization, the chamber was darkened, and postillumination isoprene release was measured for 150–200 s until the isoprene emission decayed to baseline level (Supporting Information, Fig. S1a). Before integration to determine the total DMADP pool size and the remaining pool size at given time t, isoprene signal was corrected for the empty chamber response as detailed in Rasulov *et al*. (2009a). Due to small chamber size of the Walz leaf chamber, the chamber correction was minor, less than 10% of total DMADP pool size (data not shown, see Niinemets, 2012 for comparison of chambers with different sizes and flow rates). The IspS rate constant (K) was found as the slope of the linear relationship between paired values of $I(t)$ and remaining DMADP pool size at time t passing the origin (Supporting Information, Fig. S1b).

Measurements of light and CO_2 response curves of net assimilation and isoprene emission

The leaf for the response curve measurements was stabilized under baseline conditions of photosynthetic quantum flux density of 500 μmol m^{-2} s^{-1}, leaf temperature of 30 °C, cuvette humidity of 60%, and CO_2 concentration of 380 μmol mol^{-1} for ambient-[CO_2]-grown and 780 μmol mol^{-1} for elevated-[CO_2]-grown plants. When the steady-state rates of net assimilation and isoprene emission were achieved, the CO_2 response curve was measured using the following sequence of chamber CO_2 concentrations (C_a, μmol mol^{-1}): 380→200→150→100→50→20→0→380→780→1000→1500→2000 for ambient-[CO_2]-grown plants, and 780→380→200→150→100→50→20→0→780→1000→1500→2000 for elevated-[CO_2]-grown plants. At every C_a, the values of A, I, and stomatal conductance (g_s) were recorded when the gas-exchange rates were stable, typically 5–10 min after the change of C_a.

Light (Q) response curves for every leaf were measured both at chamber CO_2 concentrations of 380 and 780 μmol mol^{-1}. After the leaf had stabilized under the baseline conditions (for light-response curves measured under C_a of 780 μmol mol^{-1}, this was also the baseline CO_2 concentration), Q (μmol m^{-2} s^{-1}) was changed in the order: 500→ 1500→ 1000→ 800→ 400→ 200→ 120→ 60→ 30→ 12→ 0. At each light level, the estimates were recorded after the steady-state values were observed, typically in ca. 10 min after change of the light level, except for the measurement at the highest light level of 1500 μmol m^{-2} s^{-1}. At this Q, the values were recorded in 5–8 min after increase of light to avoid development of photoinhibition. The net assimilation rate (A), stomatal conductance, and intercellular CO_2 concentration (C_i) were calculated according to von Caemmerer & Farquhar (1981).

Fitting of CO_2 and light response curves of net assimilation and isoprene emission rates

Farquhar *et al*. (1980) photosynthesis model was used to fit A vs. C_i response curves by a nonlinear least squares fitting procedure (Fig. 2a for sample fits), and the maximum carboxylase activity of Rubisco (V_{cmax}) and the capacity for photosynthetic electron transport (J_{max}) were derived. In the fitting, Rubisco kinetic characteristics at the measurement temperature of 30 °C were derived using the temperature relationships in Niinemets & Tenhunen (1997).

A non-rectangular hyperbola was used to fit the net assimilation vs. photosynthetic quantum flux density (Q) responses (Thornley, 1976; Ögren & Evans, 1993):

$$A = \frac{\Phi Q + A_{max} - \sqrt{(\Phi Q + A_{max})^2 - 4 \cdot \Phi Q k A_{max}}}{2k} - R_{dk} \quad (1)$$

where Φ is the initial quantum yield for the incident light, A_{max} is the light-saturated A, and R_{dk} is the dark respiration rate, and k is the curvature that characterizes how dA/dQ changes with increasing light at intermediate and high Q values, i.e., how rapidly A approaches A_{max} with increasing Q (Fig. 2b for sample fits). The initial quantum yield for photosynthetic electron transport (Φ_J) was calculated from the quantum yield of CO_2 fixation Φ as (Kellomäki & Wang, 1997):

$$\Phi = \frac{\partial A}{\partial Q}\bigg|_{Q\to 0} = \Phi_J \frac{C_i - \Gamma*}{4C_i + 8\Gamma*} \Leftrightarrow \Phi_J = \frac{\Phi(4C_i + 8\Gamma*)}{C_i - \Gamma*} \quad (2)$$

where Γ* is the hypothetical CO_2 compensation point in the absence of mitochondrial respiration that depends on Rubisco kinetic characteristics and oxygen concentration.

Recently, models based on certain key assumptions on biochemistry of isoprene synthesis have been developed to describe the CO_2 response of isoprene emission (Wilkinson *et al*., 2009; Possell & Hewitt, 2011). These models have provided satisfactory fits to declining isoprene emission rates at higher ambient CO_2 concentrations (Wilkinson *et al*., 2009; Possell & Hewitt, 2011). However, these models did not consider that the response of I to C_i is a curve with a maximum (Loreto & Sharkey, 1990; Rasulov *et al*., 2009b) and did not include a mechanism to describe the reduction of I at lower ambient CO_2 concentrations starting from ambient CO_2 concentration of ca. 150–250 μmol mol^{-1}. Furthermore, the CO_2-dependence of isoprene emission is strongly asymmetric (Loreto & Sharkey, 1990; Rasulov *et al*., 2009b). Several empirical nonlinear functions were tested to describe I vs. C_i responses, and the best fit (highest r^2) was obtained using a modified Weibull type response function (e.g. Yang *et al*., 1978) with four empirical parameters (Fig. 2c). Taking the first derivative of the function used and revealing C_i from $dI/dC_i = 0$

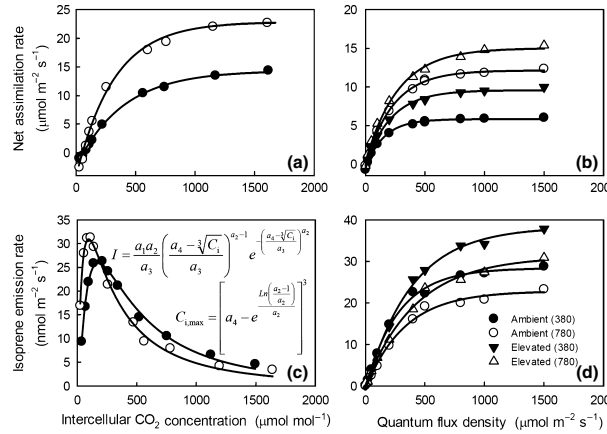

Fig. 2 Sample CO_2 (a, c) and light (b, d) responses of net assimilation (a, b) and isoprene emission rates (c, d) of representative leaves from ambient- and elevated-[CO_2]- grown plants of hybrid aspen. All measurements were conducted at the growth leaf temperature of 30 °C. The CO_2 response curves were measured under growth light of 500 μmol m^{-2} s^{-1}, and the light response curves were measured both at chamber CO_2 concentrations of 380 μmol mol^{-1} and 780 μmol mol^{-1}. The inset in (c) demonstrates the modified Weibull type equation with four empirical parameters a_1–a_4 used to fit the asymmetric isoprene emission rate (I) vs. intercellular CO_2 (C_i) responses of isoprene emission. Estimation of the value of C_i corresponding to maximum isoprene emission rate ($C_{i,max}$) is also demonstrated. For maximum isoprene emission rate, $I_{max,Ci}$, C_i in the first equation was replaced by $C_{i,max}$. The lines in (a) denote nonlinear least square fits by Farquhar *et al.* (1980) photosynthesis model that assumes limitation of photosynthesis by Rubisco in low C_i and by photosynthetic electron transport in high C_i. In (b), data were fitted by Eqn (1), in (c) by equations in the inset of panel (c), and in (d) by Eqn (4).

(Niinemets, 1996), $C_{i,max}$, the C_i value for maximum isoprene emission rate ($I_{max,Ci}$) was further calculated (Fig. 2c). In addition, $I_{max,Ci}$ and I at C_i = 300 μmol mol^{-1} (I_{Ci300}) and at C_i = 500 μmol mol^{-1} (I_{Ci500}) for individual leaves measured were calculated from the parameterized functions.

Isoprene emission vs. light response curves were fitted by the light response function of Guenther *et al.* isoprene emission model (Guenther *et al.*, 1993; Niinemets *et al.*, 2010; Monson *et al.*, 2012) modified to include the light-saturated isoprene emission rate, $I_{max,Q}$:

$$I = \frac{I_{max,Q}\alpha Q}{\sqrt{1+\alpha^2 Q^2}} \quad (3)$$

where α is the apparent quantum yield of isoprene emission (Fig. 2d for sample fits, Monson *et al.*, 2012). Note that the true quantum yield, dI/dQ at $Q = 0$ is equal to $\alpha I_{max,Q}$. Solving this equation for Q = 1000 μmol m^{-2} s^{-1} provides the standardized I (I_{Q1000}), the emission factor (Guenther *et al.*, 1993; Niinemets *et al.*, 2010). The ratio, $I_{max,Q}/I_{Q1000}$ is the C_L parameter of the original Guenther *et al.* (1993) model:

$$I = \frac{I_{Q1000} C_L \alpha Q}{\sqrt{1+\alpha^2 Q^2}} \quad (4)$$

C_L characterizes the extent to which I_{Q1000} differs from the asymptotic value $I_{max,Q}$.

Foliage anatomical measurements

Two representative fully mature leaves from mid-canopy adjacent to leaves used for physiological measurements were sampled for anatomical analyses at the end of the experiment. 1 × 1 mm pieces were cut between the main veins from the middle part of the leaves, and the material was fixed by infiltrating with 2% glutaric aldehyde in 0.2 M phosphate buffer (pH 7.2) in a syringe under negative pressure. The fixed samples were dehydrated in ethanol series and embedded in Epoxy Embedding Medium (Sigma-Aldrich Chemie, Steinheim, Germany) according to Bozzola & Russell (1992). Semi-thin (0.2–0.5 μm) cross-sections for light microscopy and ultra-thin (70–90 nm) cross- and paradermal sections for transmission electron microscopy (TEM) were cut with an OM U2 Ultra Microtome (American Optical Corporation, Reichert Products, Buffalo, New York, USA) using a 45° diamond knife (Diatome, Hatfield, PA, USA). The semi-thin sections were stained for 30 s with Toluidine Blue following the protocols of Burns (1978) and Mercer (1963), and viewed using a Zeiss Axioplan 2 light microscope (Carl Zeiss Microscopy, Jena, Germany) at 400–1000x magnification. Digital images were taken with a digital camera AxioCam HRc (Carl Zeiss Microscopy, Jena, Germany). Thicknesses of the leaf, palisade, and spongy mesophyll, and upper and lower epidermis, and surface area of mesophyll cells exposed to intercellular air space per leaf area (S_m/S) were measured from the digital images

using UTHSCSA Image Tool for Windows Ver. 3.00 (UTHSCSA, Texas, USA).

The samples for TEM were further contrasted with 2% uranyl acetate in 50% ethanol solution for 2 min and in 0.2% lead citrate in 0.1 M sodium hydroxide solution for 2 min (Bozzola & Russell, 1992). The samples were viewed and photographed with a Philips Tecnai 10 TEM (FEI, Eindhoven, The Netherlands) using an accelerating voltage of 80 kV. 1000x magnification was used to measure surface area of chloroplasts exposed to intercellular air space per leaf area (S_c/S), and 2100–4200x magnification was used to measure the number and area of chloroplasts and starch grains. Calculations of S_m/S and S_c/S were conducted as in Tosens *et al.* (2012). All anatomical characteristics were estimated in 10–16 replicates per treatment.

Leaf structural and chemical analyses

After foliage physiological measurements were completed, four representative trees from each of the two treatments were harvested for structural and chemical analyses. The leaves were scanned with a resolution of 300 dpi, and leaf area was estimated from digital images with UTHSCA Imagetool 2.00alpha (The University of Texas Health Science Center, San Antonio, TX, USA). Leaf fresh mass immediately after foliage scanning and leaf dry mass after oven-drying at 65 °C for 48 h were also determined, and leaf dry to fresh mass ratio (D_F) and leaf dry mass per unit area (M_A) were calculated. Foliage nitrogen (N_M) and carbon contents (C_M) per dry mass were determined gas-chromatographically using a Vario MAX CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Leaf nitrogen (N_A) and carbon contents (C_A) per unit leaf area were also calculated.

Data analyses

Treatment effects were tested by ANOVA followed by Tukey's test whenever pertinent. Measurements of physiological traits under different CO_2 concentrations (380 μmol mol^{-1} vs. 780 μmol mol^{-1}) within treatments were compared using paired samples *t*-tests. Correlative relationships between structural, chemical, and physiological traits were tested using Pearson pairwise correlation analyses and linear regressions. The correlative relationships between treatments were compared using co-variation analyses (ANCOVA). First, a separate slope ANCOVA with the interaction term was used. When the interaction term was not statistically significant, the data were re-fitted according to a common slope ANCOVA model (Sokal & Rohlf, 1995). For all physiological and anatomical traits, at least 8–16 replicate measurements were available at each treatment. Four replicates at each treatment were available for whole plant structural and chemical traits. SPSS 17.0 (IBM SPSS Statistics) was used for all analyses, and all statistical relationships were considered significant at $P < 0.05$.

Results

Effects of growth CO_2 environment on foliage structural and chemical traits

Growth under elevated [CO_2] resulted in greater leaf dry to fresh mass ratio (D_F), dry mass per unit area (M_A), leaf thickness, mesophyll thickness, and mesophyll exposed (S_m/S) and chloroplast exposed (S_m/S) to total leaf area ratios, and greater number of chloroplasts per cell area (n_{chl}), and chloroplast area relative to cell area, demonstrating overall more developed photosynthetic mesophyll under elevated [CO_2] (Table 1). The ratio of chloroplast to exposed mesophyll surface was not affected by growth [CO_2], indicating that the chloroplasts were exposed to the same degree under different treatments.

Carbon content per dry mass (C_M) was not affected by growth [CO_2], but nitrogen content per dry mass (N_M) was somewhat lower and the amount of starch in chloroplasts was larger under elevated [CO_2] (Table 1). Nevertheless, carbon and nitrogen contents per area, the products of mass-based contents and M_A, were both greater at elevated [CO_2] (Table 1).

Leaf photosynthetic characteristics under different growth [CO_2] treatments

Net assimilation rate measured at the growth light intensity of 500 μmol m^{-2} s^{-1} and growth leaf temperature of 30 °C was similar among the treatments when measured under the same ambient [CO_2], either at 380 μmol mol^{-1} or at 780 μmol mol^{-1} (Fig. 3a) with the trend of greater net assimilation rates in elevated-[CO_2]-grown plants ($P = 0.06$ for the difference between the treatments at 380 μmol mol^{-1}). The enhancement of net assimilation rate by increasing measurement CO_2 concentration from 380 to 780 μmol mol^{-1} was not statistically different ($P = 0.2$) among the ambient-[CO_2]-grown (on average ± SE of 1.829 ± 0.036 fold increase) and elevated-[CO_2]-grown plants (1.71 ± 0.10 fold increase).

Farquhar *et al.* (1980) model parameters, the maximum carboxylase activity of Rubisco (V_{cmax}), and the capacity for photosynthetic electron transport (J_{max}) per unit area, were both larger in elevated-[CO_2]-grown plants, demonstrating greater foliage biochemical photosynthesis potentials (Table 2a, Fig. 4a). However, the mass-based V_{cmax} (mean ± SE = 1.37 ± 0.11 μmol g^{-1} s^{-1} for ambient- and 1.14 ± 0.08 μmol g^{-1} s^{-1} for elevated-[CO_2]-grown plants) and J_{max} (2.62 ± 0.24 μmol g^{-1} s^{-1} for ambient- and 2.12 ± 0.13 μmol g^{-1} s^{-1} for elevated-[CO_2]-grown plants) were not significantly different among the treatments ($P > 0.08$). Thus, the

Table 1 Effects of plant growth CO_2 environment, ambient (380 μmol mol^{-1}) vs. elevated (780 μmol mol^{-1}) on average ± SE values of leaf morphological, chemical, and anatomical traits*

Trait	Treatment		P§
	Ambient	Elevated	
Dry to fresh mass ratio (*DF*, %)	23.1 ± 1.0	27.10 ± 0.34	0.009
Leaf dry mass per unit area (M_A, g m^{-2})	28.8 ± 0.6	38.6 ± 0.8	<0.001
Leaf carbon content per dry mass (C_M, %)	37.4 ± 0.6	39.2 ± 1.2	0.07
Leaf carbon content per leaf area (C_A, g m^{-2})	10.76 ± 0.30	14.91 ± 0.36	<0.001
Nitrogen content per dry mass (N_M, %)	4.21 ± 0.08	3.36 ± 0.05	<0.001
Nitrogen content per leaf area (N_A, g m^{-2})	1.211 ± 0.019	1.295 ± 0.026	0.04
Leaf thickness (μm)	138.0 ± 3.1	160.1 ± 2.9	<0.001
Mesophyll thickness (μm)	113.2 ± 3.0	137.0 ± 2.8	<0.001
Chloroplast number per 100 μm^2 cell area (n_{chl})	2.27 ± 0.11	2.94 ± 0.11	<0.001
Chloroplast area from cell area (%)	20.9 ± 2.3	28.4 ± 2.4	0.03
Percentage chloroplast area covered by starch granules (%)	35.5 ± 4.2	54.2 ± 4.5	<0.001
Surface area of chloroplasts exposed to intercellular air space per leaf area (S_c/S, m^2 m^{-2})	11.4 ± 0.8	13.7 ± 0.8	0.04
Surface area of mesophyll cells exposed to intercellular air space per leaf area (S_m/S, m^2 m^{-2})	17.3 ± 0.8	20.6 ± 0.8	0.005
Chloroplast to mesophyll exposed surface area ratio (S_c/S_m, m^2 m^{-2})	0.650 ± 0.020	0.661 ± 0.020	0.8

*Data are mean ± SE of four independent samples (trees).
§Means were compared using ANOVA.

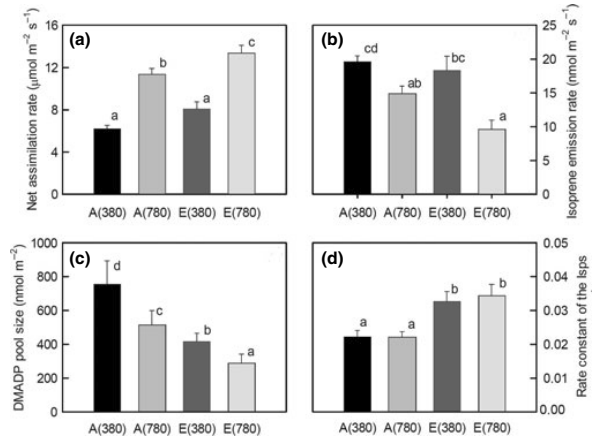

Fig. 3 Effects of growth and measurement CO_2 environments on net assimilation rate (a), isoprene emission rate (b), DMADP pool size (c), and the rate constant of the IspS reaction (d) in hybrid aspen leaves. All measurements were conducted under growth leaf temperature of 30 °C, and growth light intensity of 500 μmol m^{-2} s^{-1}. Capital letters denote growth CO_2 concentration, A for the ambient CO_2 concentration of 380 μmol mol^{-1} and E for elevated CO_2 concentration of 780 μmol mol^{-1}. The values in parentheses correspond to measurement CO_2 concentration (μmol mol^{-1}). Data are averages (± SE) of 8–10 replicate leaves. Averages with different letters are significantly different at $P < 0.05$ (ANOVA followed by Tukey's test for treatment differences and paired samples *t*-tests for differences among CO_2 measurement concentrations within treatments).

Table 2 Average ± SE characteristics of net assimilation (A) and isoprene emission rate (I) vs. intercellular CO_2 concentration (C_i) response curves: parameters of the Farquhar *et al.* (1980) photosynthesis model (a) and characteristics derived from empirical fits of I vs. C_i (b, Fig. 2c)

Characteristic	Treatment		P
	Ambient	Elevated	
(a): Photosynthetic characteristics			
Maximum carboxylase activity of Rubisco (V_{cmax}, μmol m^{-2} s^{-1})	39.3 ± 2.0	44.8 ± 1.6	0.03
Capacity for photosynthetic electron transport (J_{max}, μmol m^{-2} s^{-1})	70.1 ± 2.7	87 ± 6	0.02
J_{max}/V_{cmax} (μmol electrons (μmol CO_2)$^{-1}$)	1.80 ± 0.06	1.96 ± 0.07	0.08
Mitochondrial respiration rate (R_d, μmol m^{-2} s^{-1})	0.65 ± 0.09	0.52 ± 0.07	0.2
(b): Isoprene emission characteristics			
Intercellular CO_2 concentration for maximum isoprene emission rate (Fig. 2c, $C_{i,max}$, μmol mol^{-1})	154.5 ± 3.3	96 ± 5	<0.001
Isoprene emission rate at $C_{i,max}$ ($I_{max,Ci}$, nmol m^{-2} s^{-1})	21.9 ± 2.0	28.1 ± 3.4	0.02
Isoprene emission rate at C_i = 300 μmol mol^{-1} (I_{Ci300}, nmol m^{-2} s^{-1})	17.4 ± 1.1	14.0 ± 1.0	0.002
Isoprene emission rate at C_i = 500 μmol mol^{-1} (I_{Ci300}, nmol m^{-2} s^{-1})	13.2 ± 0.9	10.0 ± 0.8	0.009

The measurements were conducted at an incident quantum flux density of 500 μmol m^{-2} s^{-1}. Data are averages ± SE of 8–10 replicates at each treatment. Means were compared using ANOVA.

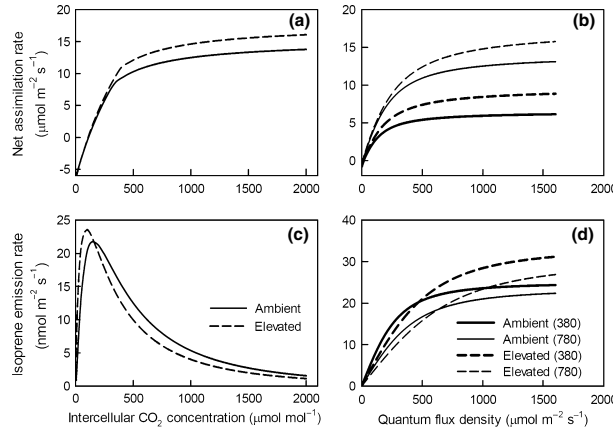

Fig. 4 Simulated net assimilation (a, b) and isoprene emission rate (c, d) responses to intercellular CO_2 concentration (a, c) and to quantum flux density (b, d) in hybrid aspen plants grown under ambient (380 μmol mol^{-1}, solid lines) and elevated (780 μmol mol^{-1}, dashed lines) CO_2 concentrations based on the mean fitted parameters (Tables 2 and 3). In the simulations, leaf temperature was set to growth leaf temperature of 30 °C, and the CO_2 responses were simulated at growth light intensity of 500 μmol m^{-2} s^{-1}, and the light responses at measurement CO_2 concentrations of 380 μmol mol^{-1} (thick lines) and 780 μmol mol^{-1} (thin lines).

enhancement of area-based photosynthetic potentials, the products of mass-based potentials and leaf dry mass per unit area, mainly reflected enhanced foliage structural development under high growth CO_2 (Table 1).

Important differences in the light response curve parameters (Eqns (1) and (2)) were observed across the treatments. In particular, elevated-[CO_2]-grown plants had significantly higher initial quantum yield for CO_2 at the measurement [CO_2] of 380 μmol mol^{-1} (Table 3a, Fig. 4b), and greater quantum yield for photosynthetic electron transport (Eqn (2)) at both measurement CO_2 concentrations (Table 3a). However, ambient-[CO_2]-grown plants had greater curvature at both measurement CO_2 concentrations (Table 3a), and the light-saturated value of net assimilation rate (A_{max}) was similar among the [CO_2] treatments, although with the trend of

Table 3 Average ± SE parameters of net assimilation (a) and isoprene emission (b) rate vs. light (Q) response curves in plants grown under ambient and elevated [CO_2] and measured at two different CO_2 concentrations

Characteristic	Treatment (measurement CO_2 concentration, μmol mol^{-1})			
	Ambient (380)	Ambient (780)	Elevated (380)	Elevated (780)
(a): Parameters of net assimilation (A) vs. light response (Eqns (1) and (2))				
Light-saturated A (A_{max}, μmol m^{-2} s^{-1})	7.1 ± 0.9^{a}	14.5 ± 1.4^{bc}	10.3 ± 1.1^{ab}	17.8 ± 1.4^{c}
Initial quantum yield for CO_2 (Φ, mol mol^{-1})	0.0441 ± 0.0038^{a}	0.0547 ± 0.0023^{b}	0.0560 ± 0.0019^{b}	0.0633 ± 0.0030^{c}
Initial quantum yield for photosynthetic electron transport (Φ_J, mol mol^{-1})	0.272 ± 0.019^{a}	0.271 ± 0.011^{a}	0.341 ± 0.012^{b}	0.316 ± 0.015^{b}
Dark respiration rate (R_{dk}, μmol m^{-2} s^{-1})	0.63 ± 0.17^{bc}	0.38 ± 0.18^{a}	0.84 ± 0.17^{c}	0.52 ± 0.16^{ab}
Curvature (k)	0.67 ± 0.05^{b}	0.64 ± 0.07^{b}	0.49 ± 0.05^{a}	0.47 ± 0.06^{a}
(b): Parameters of isoprene emission (I) vs. light response (Eqns (3) and (4))				
Light-saturated I ($I_{max,Q}$, nmol m^{-2} s^{-1})	25.8 ± 2.0^{a}	22.6 ± 1.7^{a}	38.5 ± 3.2^{b}	35.8 ± 2.9^{b}
I at Q = 1000 (I_{Q1000}, nmol m^{-2} s^{-1})	23.8 ± 2.3^{b}	19.8 ± 1.7^{a}	30.0 ± 2.6^{c}	24.2 ± 3.0^{ab}
$I_{max,Q}/I_{Q1000}$ (C_L)	1.101 ± 0.021^{a}	1.31 ± 0.06^{bc}	1.152 ± 0.019^{ab}	1.40 ± 0.07^{c}
Apparent quantum yield (α, m^2 s μmol^{-1})	0.00282 ± 0.00021^{c}	0.00204 ± 0.00026^{b}	0.00157 ± 0.00014^{b}	0.00109 ± 0.00011^{a}
True quantum yield (α_T, mmol mol^{-1})	0.063 ± 0.005^{c}	0.0372 ± 0.0034^{ab}	0.050 ± 0.005^{bc}	0.02922 ± 0.0046^{a}
Ratio α_T/Φ_J (mmol mol^{-1})	0.259 ± 0.029^{c}	0.154 ± 0.017^{b}	0.149 ± 0.019^{b}	0.099 ± 0.011^{a}

Data are means ± SE of 8–10 independent replicates. The means followed by different letters are significantly different at $P < 0.05$ (ANOVA followed by Tukey's test for treatment differences and paired samples t-tests for differences among measurement CO_2 concentrations within treatment).

higher rate at elevated-[CO_2]-grown plants (Fig. 4b, Table 3a, $P = 0.06$ for the comparison of A_{max} measurements at 380 μmol mol^{-1} and $P = 0.09$ for the comparison at 780 μmol mol^{-1}).

CO_2 treatment effects on isoprene emission, DMADP pool size and isoprene synthase rate constant under growth conditions

Growth [CO_2] did not significantly alter isoprene emission rates at the growth light intensity of 500 μmol m^{-2} s^{-1} and growth leaf temperature of 30 °C either at measurement [CO_2] of 380 μmol mol^{-1} or 780 μmol mol^{-1} (Fig. 3b). However, the inhibition of isoprene release by increasing measurement [CO_2] was significantly greater ($P = 0.03$) for elevated-[CO_2]-grown plants (on average ± SE of 0.58 ± 0.05 fold reduction of isoprene release by increasing measurement CO_2 concentration from 380 to 780 μmol mol^{-1}) than that in ambient-[CO_2]-grown plants (0.76 ± 0.05 fold reduction) assessed under growth light and temperature.

Chloroplastic DMADP pool was overall lower in plants grown under elevated [CO_2], and within the treatments, the pool was lower under measurement [CO_2] of 780 μmol mol^{-1} relative to the measurements under 380 μmol mol^{-1} (Fig. 3c). Isoprene synthase rate constant (K) that characterizes the isoprene synthase (IspS) activity, did not depend on measurement [CO_2], but growth under elevated [CO_2] resulted in greater IspS activity (Fig. 3d).

Isoprene emission rate was positively correlated with DMADP pool size for plants grown both under elevated and ambient [CO_2], but the slope of this relationship was greater for plants grown under elevated [CO_2] (Fig. 5, $P < 0.001$). In elevated-[CO_2]-grown plants, isoprene emission rate was positively correlated with K

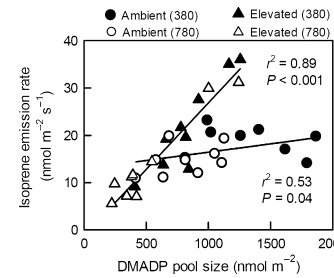

Fig. 5 Correlations of leaf isoprene emission rate with DMADP pool size in hybrid aspen plants under different growth CO_2 concentrations of 380 μmol mol^{-1} (ambient) and 780 μmol mol^{-1} (elevated) and measurement CO_2 conditions (380 vs. 780 μmol mol^{-1}). All measurements were conducted at growth leaf temperature of 30 °C and growth quantum flux density at 500 μmol m^{-2} s^{-1}. Data were fitted separately for different CO_2 growth environments by linear regressions.

($r^2 = 0.41$, $P < 0.01$ for measurements at $[CO_2]$ of 380 μmol mol^{-1} and 780 μmol mol^{-1} pooled), but I did not correlate with K for plants grown under ambient $[CO_2]$ ($r^2 = 0.11$, $P > 0.2$). Nevertheless, for all data pooled, I was correlated positively with both the DMADP pool size and K ($P < 0.01$ for both variables). According to multiple regression analyses, both DMADP and K significantly affected I in ambient-$[CO_2]$-grown ($r^2 = 0.77$, $P < 0.001$) and elevated-$[CO_2]$-grown plants ($r^2 = 0.83$, $P < 0.001$).

CO_2 response curves of isoprene emission in relation to growth $[CO_2]$

Analysis of isoprene emission rate (I) vs. intercellular CO_2 (C_i) response curves (Fig. 2c) demonstrated that the maximum isoprene emission rate ($I_{max,Ci}$) was higher in elevated-$[CO_2]$-grown plants (Table 2b, Fig. 4c). Furthermore, the shape of I vs. C_i was importantly affected by plant growth CO_2 concentration (Table 2b, Fig. 4c). In particular, the C_i corresponding to $I_{max,Ci}$ ($C_{i,max}$, Fig. 2c) was significantly lower in elevated-$[CO_2]$-grown plants (Fig. 2c, 4c and Table 2b). Despite higher $I_{max,Ci}$, the shift in $C_{i,max}$ implied that after reaching the same emission value at ca. C_i = 150 μmol mol^{-1}, the emission rate of elevated-$[CO_2]$-grown plants tended to be somewhat lower at higher CO_2 concentrations (Fig. 4c, Table 2b for comparisons of the emission rates predicted according to the fitted empirical model (Fig. 2c) at C_i = 300 μmol mol^{-1} and 500 μmol mol^{-1}).

The values of $C_{i,max}$ were negatively correlated with the ratio of $I_{max,Ci}$ to the emission rate measured at chamber CO_2 concentration of 380 μmol mol^{-1} ($I_{max,Ci}/I_{380}$) and positively with the DMADP pool size (Fig. 6). Although the correlation between $C_{i,max}$ and $I_{max,Ci}/I_{380}$ was not significant for ambient-$[CO_2]$-grown plants where the range of variation was small, the regression was highly significant when data from both ambient and elevated CO_2 were pooled ($r^2 = 0.90$, $P < 0.001$).

Modification of light responses of isoprene emission by growth $[CO_2]$

Light-response curves further demonstrated important differences in isoprene emission characteristics among the treatments. The light-saturated isoprene emission rate, $I_{max,Q}$, was greater under both measurement CO_2 concentrations of 380 and 780 μmol mol^{-1} in plants grown under elevated $[CO_2]$ (Table 3b, Fig. 4d). As isoprene emission rates at the growth light intensity of 500 μmol m^{-2} s^{-1} did not differ at given measurement CO_2 concentration among ambient- and elevated-$[CO_2]$-grown plants (Fig. 3b), this suggests a cross-over of emission light responses at greater light. Such cross-over was observed typically at light intensities between 500 and 700 μmol m^{-2} s^{-1} (Fig. 4d).

Similarly to foliage biochemical potentials, the treatment differences in area-based $I_{max,Q}$ values were importantly driven by treatment effects on leaf dry mass per unit area (Table 1). Mass-based $I_{max,Q}$ values were not statistically different among the treatments under both measurement CO_2 concentrations (data not shown, $P > 0.2$).

The "true" quantum yield of isoprene emission, α_T, did not differ statistically among the treatments at both measurement CO_2 concentrations (Table 3b). However, the ratio of the quantum yields for isoprene emission and photosynthetic electron transport, α_T/Φ_J, was less in elevated-$[CO_2]$-grown plants under both measurement CO_2 concentrations (Table 3b), indicating reduced efficiency for use of photosynthetic electron transport in isoprene emission.

Measurement CO_2 concentration did not alter $I_{max,Q}$, but α_T and α_T/Φ_J were less under measurement $[CO_2]$ of 780 μmol mol^{-1} than under 380 μmol mol^{-1} in both treatments. $I_{max,Q}$ and α_T were positively correlated in three out of the four treatment x measurement $[CO_2]$ combinations (Fig. 7). Co-variation analyses (ANCOVA) indicated no differences in the slope of this relation-

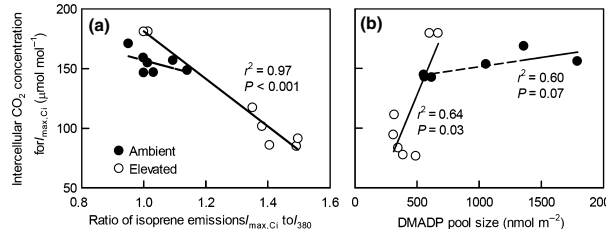

Fig. 6 Dependencies of the intercellular CO_2 concentration (C_i) for maximum isoprene emission rate ($I_{max,Ci}$) on (a) the ratio of $I_{max,Ci}$ to the isoprene emission rate measured at ambient CO_2 of 380 μmol mol^{-1} (I_{380}), and (b) on the DMADP pool size in plants grown under ambient (filled symbols) and elevated (open symbols) $[CO_2]$. Data were fitted by linear regressions ($P > 0.2$ for the non-significant regression for ambient-$[CO_2]$-grown plants in (a)).

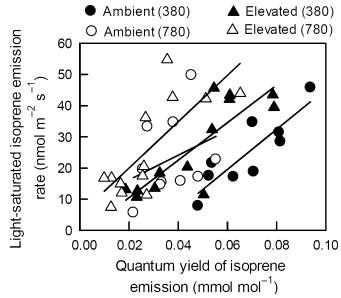

Fig. 7 Correlation between the light-saturated isoprene emission rate, $I_{max,Q}$, and the true quantum yield for isoprene emission, α_T, in hybrid aspen plants grown either at CO_2 concentration of 380 μmol mol^{-1} or 780 μmol mol^{-1} and measured both at 380 μmol mol^{-1} (A380 for ambient-[CO_2]-grown and E380 for elevated-[CO_2]-grown plants) and 780 μmol mol^{-1} (A780 and E780). Data were fitted by linear regressions: $r^2 = 0.75$, $P < 0.001$ for A380; $r^2 = 0.11$, $P > 0.3$ for A780; $r^2 = 0.71$, $P < 0.001$ for E380 and $r^2 = 0.59$, $P < 0.01$ for E780. Symbols as in Fig. 5.

ship, but the intercept was significantly less for ambient-[CO_2]-grown plants measured at 380 μmol mol^{-1} than in the rest of the treatments ($P < 0.01$), and the intercept was significantly less for elevated-[CO_2]-grown plants measured at 380 μmol mol^{-1} than at 780 μmol mol^{-1} ($P < 0.05$), indicating lower $I_{max,Q}$ achieved at given α_T for these treatment x measurement [CO_2] combinations (Fig. 7).

The Guenther *et al.* (1993, 2006) model parameter, the apparent quantum yield, $\alpha = \alpha_T/I_{max,Q}$, was less in elevated-[$CO_2$]-grown plants in both treatments (Table 3b), and the isoprene emission factor (I_{Q1000}) was greater in elevated-[CO_2]-grown plants measured at [CO_2] of 380 μmol mol^{-1}. The parameters α, and I_{Q1000} decreased and the parameter C_L increased with increasing the measurement [CO_2] in both treatments (Table 3b), indicating that light-saturation of isoprene emission occurred at progressively higher light intensities (Fig. 4d).

Discussion

Structural, chemical, and photosynthetic adaptation to growth CO_2 environment

In our study, growth under elevated [CO_2] resulted in greater leaf dry mass per unit area and leaf thickness and overall more developed mesophyll with greater exposed mesophyll cell wall and chloroplast surface area (Table 1). Higher leaf thickness and more structured mesophyll under elevated [CO_2] has been observed in several other studies (e.g. Sims *et al.*, 1998a, b; Miyazawa *et al.*, 2011), and has been interpreted as enhanced supply of carbon for leaf construction, so-called morphological "upregulation" (Luo *et al.*, 1997). However, elevated [CO_2] often results in "excessive", unbalanced, carbon supply, reflected in extensive accumulation of starch and soluble carbohydrates, and in stronger N limitation for construction of photosynthetic machinery, leading to reduced foliage N content and decreased photosynthetic capacity, known as "downregulation of photosynthesis" (Luo *et al.*, 1997; Curtis & Wang, 1998; Long *et al.*, 2004; Nowak *et al.*, 2004; Johnson, 2006). Yet, the downregulation strongly depends on nutrient availability, and under optimum supply of nutrients, photosynthetic capacity may even increase under elevated [CO_2] "filling up" the mesophyll framework (Curtis, 1996; Luo *et al.*, 1997; Curtis & Wang, 1998). In our study, starch accumulation was larger under elevated [CO_2], and this was associated with decreased N content per dry mass (Table 1). However, N content per area actually increased under elevated [CO_2] (Table 1) and this resulted in enhanced photosynthetic capacity (Table 2) and greater quantum yield of photosynthesis and photosynthetic electron transport for incident light (Table 3). Furthermore, foliage photosynthetic potentials, J_{max} and V_{cmax} per unit dry mass were not affected by growth CO_2 concentration. Thus, this evidence collectively indicates that no downregulation of photosynthesis occurred in hybrid aspen plants maintained under high nutrient supply in our study.

Effects of growth CO_2 on isoprene emission capacity

Under the plant growth conditions of moderately high light of 500 μmol m^{-2} s^{-1} and leaf temperature of 30 °C, growth [CO_2] did not significantly affect isoprene emission rate (Fig. 3b). Furthermore, at CO_2 concentration yielding maximum isoprene emission rate (Fig. 2c, Fig. 4c, Table 2b) and at light saturation (Table 3b, Fig. 4d), isoprene emission rate was greater in elevated-[CO_2]-grown plants than in ambient-[CO_2]-grown plants, indicating enhanced capacity for isoprene emission. This result is in contrast to several previous observations showing reduced emission capacity under elevated compared with ambient [CO_2], but the results of our study agree with a number of past findings of either no significant change or even increased emissions (see Introduction for pertinent references).

What is common with the studies exhibiting unaltered or enhanced isoprene emission capacity under elevated growth [CO_2]? In these studies, photosynthetic capacity (light-saturated net assimilation rate standardized to a common C_i, or Farquhar *et al.* (1980) model parameters V_{cmax} and J_{max}) was not affected by ele-

vated [CO_2] or was even "upregulated" in elevated-[CO_2]-grown plants (our study, Darbah *et al.*, 2010; Rapparini *et al.*, 2004; Staudt *et al.*, 2001; Loreto *et al.*, 2001a; Calfapietra *et al.*, 2007, 2008; Sharkey *et al.*, 1991; Brilli *et al.*, 2007b). In contrast, in all studies reporting reduced isoprene emissions under elevated [CO_2], there was also downregulation in photosynthetic capacity (Darbah *et al.*, 2010 one aspen clone); (Possell *et al.*, 2004; Scholefield *et al.*, 2004; Monson *et al.*, 2007; Possell & Hewitt, 2009, 2011; Velikova *et al.*, 2009; Wilkinson *et al.*, 2009). To our knowledge, the only exception is the study of Sharkey *et al.* (1991) where the reduction in isoprene emission capacity in elevated-[CO_2]-grown aspen was associated with constant photosynthetic capacity.

The association of downregulations in photosynthetic and isoprene emission potentials does not necessarily imply a direct cause/effect linkage between the capacities for photosynthesis and isoprene emission. However, it may still suggest that similar controls operate on both processes. Such a common control can be the availability of key limiting nutrients such as N for protein synthesis that could ultimately drive modifications in enzymatic capacities for both photosynthesis and isoprene emission, thereby providing an explanation for the study-to-study variations in isoprene emissions under elevated [CO_2]. This suggestion is partly supported by the study of Possell *et al.* (2004) where fertilization reduced depression of isoprene emissions under elevated [CO_2], although the fertilization in this study was not enough to avoid downregulations of photosynthesis and isoprene emission.

Analysis of growth CO_2 effects on immediate isoprene precursor, dimethylallyldiphosphate (DMADP), and isoprene synthase activity allowed gaining insight into modifications in partial limitations of isoprene emissions. Although isoprene emission rates were not different among [CO_2] treatments at growth light intensity (Fig. 3b), this constancy was associated with major changes at cellular level. In particular, elevation of growth [CO_2] resulted in reduced DMADP pool size (Fig. 3c), but this was compensated by increases in isoprene synthase activity (Fig. 3d, Fig. 5). So far, few studies have analyzed changes in DMADP pool size vis-à-vis changes in isoprene synthase activity in plants grown under different CO_2 concentrations. In the study of Scholefield *et al.* (2004), reduced isoprene emission of *Phragmites australis* at the bottom of the CO_2 vent relative to the edge was associated with decreased isoprene synthase activity. However, no change in isoprene synthase activity at elevated [CO_2] was found in *Populus tremuloides* (Calfapietra *et al.*, 2007). In another study, Calfapietra *et al.* (2008) demonstrated reduced DMADP content in elevated-[CO_2]-grown *P. tremuloides*, but similar isoprene emission rate, suggesting enhancement of isoprene synthase activity, concurring with our observations in hybrid aspen. In Possell & Hewitt (2011) reduced isoprene emission capacity in *Acacia nigrescens* was associated with both decreased DMADP pool size and isoprene synthase activity. In common with all these observations is that when the reduction in synthase activities has been observed, there was also downregulation of photosynthetic capacity, possibly reflecting nutrient limitations under elevated [CO_2]. Differently from synthase activities, the reduction in substrate pool size in elevated-[CO_2]-grown plants seems to be a common phenomenon independent of what happens with synthase activity or photosynthetic metabolism under elevated [CO_2].

Why is isoprene release limited more strongly by DMADP pool size in elevated-[CO_2]-grown plants?

Overall, the evidence in this study suggests that growth under elevated [CO_2] resulted in greater limitation of isoprene emission by DMADP pool size than in plants grown under ambient [CO_2]. What can be responsible for lower chloroplastic DMADP pool size in elevated-[CO_2]-grown plants? There is still a debate over the origin of the intermediates for 1-deoxy-D-xylulose-phosphate/2-C-methylerythritol 5-phosphate (DOXP/MEP) pathway (Schnitzler *et al.*, 2004; Sharkey *et al.*, 2008; Monson *et al.*, 2009). Although glyceraldehyde-3-phosphate (GAP) under non-stressed conditions is considered to come directly from photosynthesis, until recently, chloroplasts were considered not to be capable of pyruvate (Pyr) formation. Thus, a necessary transport step of Pyr precursor, phosphoenolpyruvate (PEP), from cytosol into chloroplasts by PEP transporter has been postulated (Rosenstiel *et al.*, 2003; Monson *et al.*, 2009). Cytosolic PEP availability was suggested to be controlled by PEP carboxylase activity, that was supposedly more active under high [CO_2], limiting more strongly PEP transport into chloroplasts, and thereby ultimately chloroplastic DMADP pool size (Rosenstiel *et al.*, 2003; Monson *et al.*, 2009; Wilkinson *et al.*, 2009).

Labeling experiments do demonstrate that recently assimilated ^{13}C in isoprene can be traced back to both GAP and Pyr (Karl *et al.*, 2002; Trowbridge *et al.*, 2012). However, the key problem in quantitatively considering the role of cytosolic PEP in reduced isoprene emissions under elevated [CO_2] is overall lack of information on cytosolic PEP carboxylase kinetic characteristics in isoprene-emitting species. Furthermore, the evidence of growth CO_2 effects on PEP carboxylase activity is controversial. To our knowledge, only Possell & Hewitt (2011) have demonstrated enhanced PEP carboxylase activity under higher growth [CO_2] coupled to reduced isoprene

emission. In contrast, despite the reduction in isoprene emission rate under elevated [CO_2], Velikova *et al.* (2009) observed either no changes or reduced PEP carboxylase activity in different-aged leaves. Furthermore, increased PEP carboxylase activity, but constant isoprene emission under elevated [CO_2] has also been reported, with a reverse correlation between PEP carboxylase activity and isoprene emission rate observed only under ozone stress across the CO_2 treatments (Loreto *et al.*, 2007). In the labeling experiments of Trowbridge *et al.* (2012) with *Populus x canescens*, isoprene labeling kinetics were similar in both the plants grown under ambient and elevated [CO_2]. Only in plants under sub-ambient [CO_2], with heavily suppressed photosynthesis, there was evidence of greater contribution of cytosolic pyruvate to isoprene (Trowbridge *et al.*, 2012). The role of PEP carboxylase must also be reconsidered in light of evidence of widespread occurrence of a plastidial pyruvate transporter in plant kingdom, indicating that when needed, Pyr rather than PEP might be transported into chloroplasts (Furumoto *et al.*, 2011). Overall, the existing evidence collectively does not yet provide conclusive insight into the role of PEP carboxylase in regulation of isoprene emission in elevated-[CO_2]-grown plants.

On the other hand, presence of two key enzymes, phosphoglyceromutase and enolase has been recently detected in chloroplasts (Joyard *et al.*, 2010; Bayer *et al.*, 2011), implying that chloroplasts are in principle capable of synthesizing pyruvate, and that an obligatory cytosolic step of isoprene synthesis might not be necessary. In fact, early experiments of Sanadze and colleagues with isolated chloroplasts have demonstrated that cytosolic steps are not mandatory for high isoprene emission rates (Sanadze & Dzhaiani, 1972; Mgalobilishvili *et al.*, 1978). Thus, modifications in DMADP pool size may reflect alterations in chloroplastic processes. In particular, we suggest that the reduction in DMADP can result from the following: 1) overall low capacity of DOXP/MEP enzymes upstream of isoprene synthase such that the DMADP pool size is maintained low; 2) low energy status of chloroplasts limiting DMADP synthesis, and 3) competition of isoprene synthesis for DMADP by other key metabolic pathways.

Regarding the hypothesis of overall lower capacity of DOXP/MEP pathway in leaves developed under elevated [CO_2], enhanced isoprene emissions at [CO_2] optimum of isoprene emission (Fig. 2c, Fig. 4c, Table 2b) and under light saturation and (Table 3b, Fig. 4d) conclusively demonstrate that high DMADP pools can be reached in chloroplasts in elevated-[CO_2]-grown plants, suggesting that the DMADP formation capacity is not inherently low in elevated-[CO_2]-grown plants. As both the conditions of low [CO_2] and high light are associated with enhanced leaf ATP status (Rasulov *et al.*, 2009b, 2011), low ATP concentration may limit DMADP synthesis. Reduced ATP level can commonly occur in high [CO_2] as a result of inorganic phosphate sequestration into intermediates of Calvin cycle, thereby inhibiting ATP synthesis, known as feedback-limited photosynthesis (Sharkey & Vanderveer, 1989; Socias *et al.*, 1993). Such a possible limitation of DMADP synthesis should not be strictly interpreted as lack of ATP per se, as the overall requirement for energetic and reductive equivalents for isoprene synthesis is small compared with CO_2 assimilation, typically 1–2% of total electron flow (Niinemets *et al.*, 1999; Niinemets, 2004). Rather, reduction of DMADP synthesis under conditions of reduced ATP synthesis should reflect the low effective Michaelis-Menten constant for ATP of the DOXP/MEP pathway (Rasulov *et al.*, 2009b, 2011). However, the data in our study do not support the hypothesis of low energy status as an explanation of low DMADP pool size in elevated-[CO_2]-grown plants. In fact, the quantum yields for net assimilation and photosynthetic electron transport were actually higher in elevated-[CO_2]-grown plants (Table 3a). The ratio of quantum yields of isoprene emission to photosynthetic electron transport was smaller in elevated-[CO_2]-grown plants, suggesting lower efficiency of conversion of available electrons into isoprene.

Finally, competition for DMADP by other chloroplastic metabolic pathways can provide an alternative explanation for reduced DMADP pool size under elevated [CO_2]. Apart from isoprene, chloroplastic DMADP is used for synthesis of a number of essential isoprenoids, including carotenoids and the phytyl chain of chlorophylls, as well as for plastoquinone, and gibberellins (Pichersky & Gershenzon, 2002; Laule *et al.*, 2003; Lichtenthaler, 2009). Given the overall greater photosynthetic activity with no downregulation, greater demand for "essential" isoprenoids for construction of photosynthetic machinery and/or its turnover and/or for plant growth in elevated-[CO_2]-grown plants is plausible. On the other hand, enhanced rate of isoprene emission due to greater enzymatic capacity under stress such as heat stress (Darbah *et al.*, 2010) or under enhanced quantum flux densities (Table 3b) potentially resulting in photoxidative stress for prolonged exposures (Osmond *et al.*, 1999) may suggest that the enhanced isoprene synthase activity is adaptive under elevated [CO_2]. Also, maintenance of high isoprene emission capacity will keep DOXP/MEP pathway active for periods when "essential" isoprenoid demand is less, e.g. during stress, thereby providing a means for more rapid onset of "essential" isoprenoid synthesis when needed, e.g. after stress during intensive repair and during intensive photosynthesis and growth (Owen & Peñuelas, 2005; Peñuelas & Munné-Bosch, 2005). Thus, high emission capacity

can be a means to support construction and maintenance of the higher capacity photosynthetic machinery in elevated-[CO_2]-grown plants.

CO_2-responsiveness of isoprene emission in relation to growth [CO_2]

So far, very few studies have investigated the full CO_2 response curve of isoprene emission (Loreto & Sharkey, 1990; Rasulov *et al.*, 2009b). Wilkinson *et al.* (2009) reported truncated CO_2 response curves of isoprene emission from C_i of ca. 200–900 μmol mol^{-1} such that the declining part of the emission response curve below C_i of 200 μmol mol^{-1} (e.g. Fig. 2c) could not be examined. They fitted the CO_2 responses empirically using a shape of the curve derived from a model that assumed limited PEP transport into chloroplasts due to increased PEP carboxylase activity at higher [CO_2] (Wilkinson *et al.*, 2009). No change in the shape of the emission response curve between the plants grown at 400 and 600 μmol mol^{-1} was observed over the measurement [CO_2] range (Wilkinson *et al.*, 2009). However, plants grown at CO_2 concentrations of 800 and 1200 μmol mol^{-1} were characterized by lower CO_2-responsiveness, implying higher emission rates at higher measurement [CO_2] compared with the rates measured at C_i = 400 μmol mol^{-1} (Wilkinson *et al.*, 2009). This contrasts with the results in our study, where the CO_2-responsiveness was actually greater in elevated-[CO_2]-grown plants (Fig. 2b, Fig. 4c, Table 2b).

However, in the study of Wilkinson *et al.* (2009), the overall CO_2-responsiveness was small, 20–25% for the studied [CO_2] range of 200–900 μmol mol^{-1}, and actually much less than the sigmoidal response predicted by their model. Low CO_2-responsiveness has also been observed in the study of Li *et al.* (2011) in *Quercus robur*. In our study, the CO_2-responsivenss was much bigger, twofold to threefold for the corresponding range of C_i of 200–900 μmol mol^{-1} (Fig. 4c). Analogous strong CO_2-sensitivity has been previously reported for *Quercus rubra* (Loreto & Sharkey, 1990). These study-to-study differences in the emission sensitivity to instantaneous modifications in [CO_2] are currently not understood.

What can be responsible for the growth [CO_2]-driven changes in the optimum CO_2 concentration for maximum isoprene emission ($C_{i,max}$) (Table 2b)? Previous studies have conclusively demonstrated that the carbon requirement for maximum isoprene emission is small (Loreto & Sharkey, 1993; Rasulov *et al.*, 2009b), and that photosynthetic carbon sources can be replaced by chloroplastic reserves such as starch or rely on carbon export from cytosol under conditions inhibiting photosynthesis (Karl *et al.*, 2002; Wolfertz *et al.*, 2003). Nevertheless, DMADP synthesis requires ATP and reductive equivalents, and at lower [CO_2], typically below the CO_2 compensation point, photosynthetic electron transport rate, and ATP synthesis become strongly inhibited due to lack of electron acceptors (Miyake *et al.*, 2005; Kiirats *et al.*, 2009), most likely explaining the reduction of isoprene emission rate below $C_{i,max}$ (Rasulov *et al.*, 2009b). On the other hand, elevated-[CO_2]-grown plants typically are characterized by higher capacity for starch synthesis and starch accumulation (Ludewig *et al.*, 1998; Heineke *et al.*, 1999), mainly resulting from greater activity of ADP-glucose pyrophosphorylase that is positively regulated by 3-PGA (Gibson *et al.*, 2011). Activation of starch synthesis as soon as 3-PGA pool builds up in leaves conducting positive photosynthesis typically also results in suppression of chloroplastic phosphate level (Sharkey & Vanderveer, 1989). Thus, greater activity of starch synthesis in elevated-[CO_2]-grown plants may explain faster reduction of DMADP level with increasing [CO_2]. This together with overall greater isoprene synthase activity (Fig. 3d), may explain the greater isoprene emission rate at $C_{i,max}$, and shifting optimum $C_{i,max}$. This reasoning is supported by positive correlations between $C_{i,max}$ and DMADP pool size (Fig. 6b). Furthermore, close to uniform negative correlation between $C_{i,max}$ and the ratio of $I_{max,Ci}$ to I_{380} (Fig. 6a) suggests that the higher is the capacity for isoprene emission relative to DMADP pool size, the earlier isoprene emission rate starts to decrease with increasing CO_2 concentration. Clearly, more active supply of DOX/MEP pathway intermediates from cytosol in elevated-[CO_2]-grown plants can also play a role, but so far such a possibility is not supported by available evidence from labeling experiments (Trowbridge *et al.*, 2012).

From an ecological perspective, greater emission capacity in plants grown under high [CO_2] can imply greater isoprene emissions under conditions suppressing stomatal openness and reducing intercellular CO_2 concentration. Such amplified emissions have occasionally been observed in elevated-[CO_2]-grown plants under different stresses. In aspen, heat stress accompanied by low vapor pressure deficits and stomatal closure almost cancelled out the negative effect of elevated [CO_2] on isoprene emissions (Darbah *et al.*, 2010). In *Populus deltoides,* soil drought resulted in complete abolishment of high [CO_2]-caused reductions in isoprene emissions both at leaf (Pegoraro *et al.*, 2004) and ecosystem level (Pegoraro *et al.*, 2005). Thus, having higher emission capacity can allow for enhanced emissions under stress when the need for antioxidative defenses is greatest. Such rapid modifications in emission rate can be particularly relevant for recurrent stresses such as reductions in intercellular CO_2 concentration due to mid-day stomatal closure or short-term

soil water limitations. In line with this hypothesis, a significant negative correlation between isoprene emission rate and intercellular CO_2 concentration has been observed across poplar genotypes sampled during a typical hot Mediterranean summer (Guidolotti *et al*., 2011). However, we note that enhanced long-term drought results in reduction in isoprene emission capacity as well, strongly reducing plant isoprene emission rate (Brilli *et al*., 2007a; Fortunati *et al*., 2008). This may mean that the ecological significance of enhanced emission potential becomes less under severe drought.

Modification of light response curves of isoprene emission by instantaneous and growth [CO_2]

A key result of our study is that the quantum yield of isoprene emission, α_T, depends on measurement [CO_2], being less at higher [CO_2] (Table 3b, Fig. 4d). This implies that light-saturation is reached at progressively higher light intensities with increasing [CO_2]. This effect of CO_2 on light responsiveness of isoprene emission likely reflects the reduction of DMADP pool size by increased [CO_2] (Rasulov *et al*., 2009b) that can be compensated by increased light level, speeding up DMADP synthesis likely through increased chloroplastic ATP concentration.

Differently from photosynthesis, where quantum yield for absorbed light is independent of photosynthetic capacity (Ehleringer & Björkman, 1977), we observed that α_T and the light-saturated isoprene emission rate, $I_{max,Q}$, were positively correlated (Fig. 7). Few studies have investigated acclimation in α_T and $I_{max,Q}$ simultaneously under different conditions. However, upon acclimation to higher light in the canopy, analogous increase of α_T with $I_{max,Q}$ occurred in *Liquidambar styraciflua* (recalculated from Harley *et al*., 1996) and in *Quercus alba* at higher temperatures (Harley *et al*., 1997), indicating that α_T cannot generally be considered independent of $I_{max,Q}$.

Previously, it has been suggested that the light response curves of isoprene emission are entirely driven by changes in DMADP pool size (Rasulov *et al*., 2009a,b). The strong coupling between DMADP pool size and isoprene emission has been explained by high *in vivo* Michaelis-Menten constant of isoprene synthase such that the emission rate increases linearly with DMADP under DMADP-limited conditions (Rasulov *et al*., 2009a,b). However, the scaling of α_T with $I_{max,Q}$ suggests that isoprene synthase activity can importantly alter the rate of isoprene emission at given DMADP pool size both under light-limited and light-saturated conditions, and that the light response of isoprene emission, when analyzed across leaves of varying isoprene synthase activity, is actually a mixed response determined both by isoprene synthase activity and DMADP pool size (Fig. 7).

This reasoning can also provide an explanation for lower α_T at given $I_{max,Q}$ in elevated-[CO_2]-grown plants (Fig. 7). As DMADP pool size is lower in elevated-[CO_2]-grown plants (Fig. 3c), this reduces the initial isoprene emission rate at given isoprene emission capacity (Fig. 4d). However, initially lower DMADP pool size and greater isoprene synthase activity (Fig. 3c, d) also result in shifting the light-saturation point of isoprene emission to higher quantum flux densities, implying greater light responsiveness of isoprene emission at intermediate and high quantum flux densities in elevated-[CO_2]-grown plants (Fig. 4d).

As our study demonstrated, under growth conditions, a similar isoprene emission rate was achieved in ambient- and in elevated-[CO_2]-grown plants, but this was achieved by different combinations of DMADP pool size and isoprene synthase activity (Fig. 3). The data further demonstrated that growth CO_2-dependent differences in synthase activity and DMADP pool size result in major changes in responsiveness of isoprene emissions to light. This evidence collectively has major implications for modeling isoprene emissions to current and future climates.

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References

Arneth A, Niinemets Ü, Pressley S *et al.* (2007) Process-based estimates of terrestrial ecosystem isoprene emissions: incorporating the effects of a direct CO_2-isoprene interaction. *Atmospheric Chemistry and Physics*, **7**, 31–53.

Bayer RG, Stael S, Csaszar E, Teige M (2011) Mining the soluble chloroplast proteome by affinity chromatography. *Proteomics*, **11**, 1287–1299.

Behnke K, Ehlting B, Teuber M *et al.* (2007) Transgenic, non-isoprene emitting poplars don't like it hot. *The Plant Journal*, **51**, 485–499.

Bozzola JJ, Russell LD (1992) *Electron Microscopy. principles and techniques for biologists*. Jones & Bartlett Publishers, New York.

Brilli F, Barta C, Fortunati A, Lerdau M, Loreto F, Centritto M (2007a) Response of isoprene emission and carbon metabolism to drought in white poplar (*Populus alba*) saplings. *The New Phytologist*, **175**, 244–254.

Brilli F, Tricoli D, Fares S, Centritto M, Loreto F (2007b) The use of branch enclosures to asses direct and indirect effects of elevated CO_2 on photosynthesis, respiration and isoprene emission of *Populus alba* leaves. *Forest@*, **40**, 60–68.

Buckley PT (2001) Isoprene emissions from a Florida scrub oak species grown in ambient and elevated carbon dioxide. *Atmospheric Environment*, **35**, 631–634.

Burns WA (1978) Thick sections: technique and applications. In *Diagnostic electron microscopy*, **Vol 1** (eds Trump BF, Jones RJ), pp. 146–178. Wiley, New York.

von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, **153**, 376–387.

Calfapietra C, Wiberley AE, Falbel TG *et al.* (2007) Isoprene synthase expression and protein levels are reduced under elevated O_3 but not under elevated CO_2 (FACE) in field-grown aspen trees. *Plant, Cell and Environment*, **30**, 654–661.

Calfapietra C, Scarascia Mugnozza G, Karnosky DF, Loreto F, Sharkey TD (2008) Isoprene emission rates under elevated CO_2 and O_3 in two field-grown aspen clones differing in their sensitivity to O_3. *The New Phytologist*, **179**, 55–61.

Claeys M, Graham B, Vas G *et al.* (2004) Formation of secondary organic aerosols through photooxidation of isoprene. *Science*, **303**, 1173–1176.

Curtis PS (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant, Cell and Environment*, **19**, 127–137.

Curtis PS, Wang X (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia*, **113**, 299–313.

Darbah JNT, Sharkey TD, Calfapietra C, Karnosky DF (2010) Differential response of aspen and birch trees to heat stress under elevated carbon dioxide. *Environmental Pollution*, **158**, 1008–1014.

Ehleringer J, Björkman O (1977) Quantum yields for CO_2 uptake in C_3 and C_4 plants. Dependence on temperature, CO_2 and O_2 concentration. *Plant Physiology*, **59**, 86–90.

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta*, **149**, 78–90.

Fehsenfeld F, Calvert J, Fall R *et al.* (1992) Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry. *Global Biogeochemical Cycles*, **6**, 389–430.

Fortunati A, Barta C, Brilli F, Centritto M, Zimmer I, Schnitzler J-P, Loreto F (2008) Isoprene emission is not temperature-dependent during and after severe drought-stress: a physiological and biochemical analysis. *The Plant Journal*, **55**, 687–697.

Furumoto T, Yamaguchi T, Ohshima-Ichie Y *et al.* (2011) A plastidial sodium-dependent pyruvate transporter. *Nature*, **476**, 472–475.

Ghirardo A, Gutknecht J, Zimmer I, Brüggemann N, Schnitzler J-P (2011) Biogenic volatile organic compound and respiratory CO_2 emissions after ^{13}C-labeling: online tracing of C translocation dynamics in poplar plants. *PLoS ONE*, **6**, e17393.

Gibson K, Park J-S, Nagai Y *et al.* (2011) Exploiting leaf starch synthesis as a transient sink to elevate photosynthesis, plant productivity and yields. *Plant Science*, **181**, 275–281.

Grote R, Niinemets Ü (2008) Modeling volatile isoprenoid emission – a story with split ends. *Plant Biology*, **10**, 8–28.

Guenther AB, Zimmerman PR, Harley PC, Monson RK, Fall R (1993) Isoprene and monoterpene emission rate variability: model evaluations and sensitivity analyses. *Journal of Geophysical Research*, **98**, 12609–12617.

Guenther A, Karl T, Harley P, Wiedinmyer C, Palmer PI, Geron C (2006) Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature). *Atmospheric Chemistry and Physics*, **6**, 3181–3210.

Guidolotti G, Calfapietra C, Loreto F (2011) The relationship between isoprene emission, CO_2 assimilation and water use efficiency across a range of poplar genotypes. *Physiologia Plantarum*, **142**, 297–304.

Harley P, Guenther A, Zimmerman P (1996) Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (*Liquidambar styraciflua*) leaves. *Tree Physiology*, **16**, 25–32.

Harley P, Guenther A, Zimmerman P (1997) Environmental controls over isoprene emission in deciduous oak canopies. *Tree Physiology*, **17**, 705–714.

Heald CL, Wilkinson MJ, Monson RK, Alo CA, Wang G, Guenther A (2009) Response of isoprene emission to ambient CO_2 changes and implications for global budgets. *Global Change Biology*, **15**, 1127–1140.

Heineke D, Kauder F, Frommer W, Kühn C, Gillissen B, Ludewig F, Sonnewald U (1999) Application of transgenic plants in understanding responses to atmospheric change. *Plant, Cell and Environment*, **22**, 623–628.

Johnson DW (2006) Progressive N limitation in forests: review and implications for long-term responses to elevated CO_2. *Ecology*, **87**, 64–75.

Joyard J, Ferro M, Masselon C, Seigneurin-Berny D, Salvi D, Garin J, Rolland N (2010) Chloroplast proteomics highlights the subcellular compartmentation of lipid metabolism. *Progress in Lipid Research*, **49**, 128–158.

Karl T, Fall R, Rosenstiel TN, Prazeller P, Larsen B, Seufert G, Lindinger W (2002) On-line analysis of the $^{13}CO_2$ labeling of leaf isoprene suggests multiple subcellular origins of isoprene precursors. *Planta*, **215**, 894–905.

Kellomäki S, Wang K-Y (1997) Effects of elevated O_3 and CO_2 concentrations on photosynthesis and stomatal conductance in Scots pine. *Plant, Cell and Environment*, **20**, 995–1006.

Kiirats O, Cruz JA, Edwards GE, Kramer MD (2009) Feedback limitation of photosynthesis at high CO_2 acts by modulating the activity of the chloroplast ATP synthase. *Functional Plant Biology*, **36**, 893–901.

Kroll JH, Ng NL, Murphy SM, Flagan RC, Seinfeld JH (2006) Secondary organic aerosol formation from isoprene photooxidation. *Environmental Science and Technology*, **40**, 1869–1877.

Laisk A, Oja V (1998) *Dynamics of leaf photosynthesis: rapid-response measurements and their interpretations*. CSIRO Publishing, Canberra.

Laule O, Fürholz A, Chang HS *et al.* (2003) Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 6866–6871.

Li D, Chen Y, Shi Y, He X, Chen X (2009) Impact of elevated CO_2 and O_3 concentrations on biogenic volatile organic compounds emissions from *Ginkgo biloba*. *Bulletin of Environmental Contamination and Toxicology*, **82**, 473–477.

Li Z, Ratliff EA, Sharkey TD (2011) Effect of temperature on postillumination isoprene emission in oak and poplar. *Plant Physiology*, **155**, 1037–1046.

Lichtenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**, 47–65.

Lichtenthaler HK (2009) Biosynthesis and accumulation of isoprenoid carotenoids and chlorophylls and emission of isoprene by leaf chloroplasts. *Bulletin of the Georgian National Academy of Science*, **3**, 81–94.

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants face the future. *Annual Review of Plant Biology*, **55**, 591–628.

Loreto F, Sharkey TD (1990) A gas-exchange study of photosynthesis and isoprene emission in *Quercus rubra* L. *Planta*, **182**, 523–531.

Loreto F, Sharkey TD (1993) On the relationship between isoprene emission and photosynthetic metabolites under different environmental conditions. *Planta*, **189**, 420–424.

Loreto F, Fischbach RJ, Schnitzler J-P, Ciccioli P, Calfapietra C, Baraldi R, Rapparini F (2001a) Isoprenoid emission by forest trees grown at elevated CO_2 concentrations. In *Forest research: a challenge for an integrated European approach* (ed Radoglou K), pp. 175–178. NAGREF, Thessaloniki, Greece.

Loreto F, Mannozzi M, Maris C, Nascetti P, Ferranti F, Pasqualini S (2001b) Ozone quenching properties of isoprene and its antioxidant role in leaves. *Plant Physiology*, **126**, 993–1000.

Loreto F, Centritto M, Barta C, Calfapietra C, Fares S, Monson RK (2007) The relationship between isoprene emission rate and dark respiration rate in white poplar (*Populus alba* L.) leaves. *Plant, Cell and Environment*, **30**, 662–669.

Ludewig F, Sonnewald U, Kauder F *et al.* (1998) The role of transient starch in acclimation to elevated atmospheric CO_2. *FEBS Letters*, **429**, 147–151.

Luo Y, Sims DA, Griffin KL (1997) Nonlinearity of photosynthetic responses to growth in rising atmospheric CO_2: an experimental and modelling study. *Global Change Biology*, **3**, 173–183.

Mercer EH (1963) A scheme for section staining in electron microscopy. *Journal of the Royal Microscopical Society*, **81**, 179–186.

Mgalobilishvili MP, Khetsuriani ND, Kalandadze AN, Sanadze GA (1978) Localization of isoprene biosynthesis in poplar leaf chloroplasts. *Fiziologiya Rastenii*, **25**, 1055–1061.

Miyake C, Miyata M, Shinzaki Y, Tomizawa K-I (2005) CO_2 response of cyclic electron flow around PSI (CEF-PSI) in tobacco leaves - relative electron fluxes through PSI and PSII determine the magnitude of non-photochemical quenching (NPQ) of Chl fluorescence. *Plant and Cell Physiology*, **46**, 629–637.

Miyazawa S-I, Warren CR, Turpin DH, Livingston NJ (2011) Determination of the site of CO_2 sensing in poplar: is the area-based N content and anatomy of new leaves determined by their immediate CO_2 environment or by the CO_2 environment of mature leaves? *Journal of Experimental Botany*, **62**, 2787–2796.

Monson RK, Fall R (1989) Isoprene emission from aspen leaves. Influence of environment and relation to photosynthesis and photorespiration. *Plant Physiology*, **90**, 267–274.

Monson RK, Trahan N, Rosenstiel TN *et al.* (2007) Isoprene emission from terrestrial ecosystems in response to global change: minding the gap between models and observations. *Philosophical Transactions of the Royal Society of London A*, **365**, 1677–1695.

Monson RK, Wilkinson MJ, Monson ND, Trahan N, Lee S, Rosenstiel TR, Fall R (2009) Biochemical control on the CO_2 response of leaf isoprene emisson: an alternative view of Sanadze's double carboxylation scheme. *Bulletin of the Georgian National Academy of Science*, **3**, 95–105.

Monson RK, Grote R, Niinemets Ü, Schnitzler J-P (2012) Tansley review. Modeling the isoprene emission rate from leaves. *The New Phytologist*, **195**, 541–559.

Niinemets Ü (1996) Changes in foliage distribution with tree size and relative irradiance: differences between the saplings of *Acer platanoides* and *Quercus robur*. *Ecological Research*, **11**, 269–281.

Niinemets Ü (2004) Costs of production and physiology of emission of volatile leaf isoprenoids. In *Advances in Plant Physiology*, **Vol 7** (ed Hemantaranjan A), pp. 233–268. Scientific Publishers, Jodhpur.

Niinemets Ü (2010) A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. *Ecological Research*, **25**, 693–714.

Niinemets Ü (2012) Whole plant photosynthesis. In *Terrestrial photosynthesis in a changing environment. The molecular, physiological and ecological bases of photosynthesis driving its response to the environmental changes* (eds Flexas J, Loreto F, Medrano H), pp. 403–428. Cambridge University Press, Cambridge.

Niinemets Ü, Tenhunen JD (1997) A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. *Plant, Cell and Environment*, **20**, 845–866.

Niinemets Ü, Tenhunen JD, Harley PC, Steinbrecher R (1999) A model of isoprene emission based on energetic requirements for isoprene synthesis and leaf photosynthetic properties for *Liquidambar* and *Quercus*. *Plant, Cell and Environment*, **22**, 1319–1336.

Niinemets Ü, Monson RK, Arneth A *et al.* (2010) The leaf-level emission factor of volatile isoprenoids: caveats, model algorithms, response shapes and scaling. *Biogeosciences*, **7**, 1809–1832.

Nowak RS, Ellsworth DS, Smith SD (2004) Functional responses of plants to elevated atmospheric CO_2 - do photosynthetic and productivity data from FACE experiments support early predictions? *The New Phytologist*, **162**, 253–280.

Ögren E, Evans JR (1993) Photosynthetic light-response curves. 1. The influence of CO_2 partial pressure and leaf inversion. *Planta*, **189**, 182–190.

Osmond CB, Anderson JM, Ball MC, Egerton JG (1999) Compromising efficiency: the molecular ecology of light-resource utilization in plants. In *Physiological plant ecology*. The 39th Symposium of the British Ecological Society held at the University of York, 7-9 September 1998 (eds Press MC, Scholes JD, Barker MG), pp. 1–24. Blackwell Science, Oxford.

Owen SM, Peñuelas J (2005) Opportunistic emissions of volatile isoprenoids. *Trends in Plant Science*, **10**, 420–426.

Pegoraro E, Rey A, Bobich EG, Barron-Gafford GA, Grieve KA, Malhi Y, Murthy R (2004) Effect of elevated CO_2 concentration and vapor pressure deficit on isoprene emission from leaves of *Populus deltoides* during drought. *Functional Plant Biology*, **31**, 1137–1147.

Pegoraro E, Rey A, Barron-Gafford G, Monson R, Malh Y, Murthy R (2005) The interacting effects of elevated atmospheric CO_2 concentration, drought and leaf-to-air vapour pressure deficit on ecosystem isoprene fluxes. *Oecologia*, **146**, 120–129.

Peñuelas J, Munné-Bosch S (2005) Isoprenoids: an evolutionary pool for photoprotection. *Trends in Plant Science*, **10**, 166–169.

Pichersky E, Gershenzon J (2002) The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Current Opinion in Plant Biology*, **5**, 237–243.

Possell M, Hewitt CN (2009) Gas exchange and photosynthetic performance of the tropical tree *Acacia nigrescens* when grown in different CO_2 concentrations. *Planta*, **229**, 837–846.

Possell M, Hewitt CN (2011) Isoprene emissions from plants are mediated by atmospheric CO_2 concentrations. *Global Change Biology*, **17**, 1595–1610.

Possell M, Heath J, Hewitt CN, Ayres E, Kerstiens G (2004) Interactive effects of elevated CO_2 and soil fertility on isoprene emissions from *Quercus robur*. *Global Change Biology*, **10**, 1835–1843.

Rapparini F, Baraldi R, Miglietta F, Loreto F (2004) Isoprenoid emission in trees of *Quercus pubescens* and *Quercus ilex* with lifetime exposure to naturally high CO_2 environment. *Plant, Cell and Environment*, **27**, 381–391.

Rasulov B, Copolovici L, Laisk A, Niinemets Ü (2009a) Postillumination isoprene emission: *in vivo* measurements of dimethylallyldiphosphate pool size and isoprene synthase kinetics in aspen leaves. *Plant Physiology*, **149**, 1609–1618.

Rasulov B, Hüve K, Välbe M, Laisk A, Niinemets Ü (2009b) Evidence that light, carbon dioxide and oxygen dependencies of leaf isoprene emission are driven by energy status in hybrid aspen. *Plant Physiology*, **151**, 448–460.

Rasulov B, Hüve K, Bichele I, Laisk A, Niinemets Ü (2010) Temperature response of isoprene emission *in vivo* reflects a combined effect of substrate limitations and isoprene synthase activity: a kinetic analysis. *Plant Physiology*, **154**, 1558–1570.

Rasulov B, Hüve K, Laisk A, Niinemets Ü (2011) Induction of a longer-term component of isoprene release in darkened aspen leaves: origin and regulation under different environmental conditions. *Plant Physiology*, **156**, 816–831.

Rosenstiel TN, Fisher AJ, Fall R, Monson RK (2002) Differential accumulation of dimethylallyl diphosphate in leaves and needles of isoprene- and methylbutenol-emitting and nonemitting species. *Plant Physiology*, **129**, 1276–1284.

Rosenstiel TN, Potosnak MJ, Griffin KL, Fall R, Monson RK (2003) Increased CO_2 uncouples growth from isoprene emission in an agriforest ecosystem. *Nature*, **421**, 256–259.

Sanadze GA, Dzhaiani GI (1972) Carbohydrate partition within isoprene molecule from photosynthetically assimilated CO_2. *Fiziologiya Rastenii*, **19**, 1082–1089.

Schnitzler JP, Graus M, Kreuzwieser J, Heizmann U, Rennenberg H, Wisthaler A, Hansel A (2004) Contribution of different carbon sources to isoprene biosynthesis in poplar leaves. *Plant Physiology*, **135**, 152–160.

Scholefield PA, Doick KJ, Herbert B, Hewitt CN, Schnitzler JP, Pinelli P, Loreto F (2004) Impact of rising CO_2 on VOC emissions: isoprene emission from *Phragmites australis* growing at elevated CO_2 in a natural carbon dioxide spring. *Plant, Cell and Environment*, **27**, 393–401.

Sharkey TD (2009) The future of isoprene research. *Bulletin of the Georgian National Academy of Sciences*, **3**, 106–113.

Sharkey TD, Singsaas EL (1995) Why plants emit isoprene. *Nature*, **374**, 769.

Sharkey TD, Vanderveer PJ (1989) Stromal phosphate concentration is low during feedback limited photosynthesis. *Plant Physiology*, **91**, 679–684.

Sharkey TD, Loreto F, Delwiche CF (1991) High carbon dioxide and sun/shade effects on isoprene emission from oak and aspen tree leaves. *Plant, Cell and Environment*, **14**, 333–338.

Sharkey TD, Wiberley AE, Donohue AR (2008) Isoprene emission from plants: why and how. *Annals of Botany*, **101**, 5–18.

Sims DA, Seemann JR, Luo Y (1998a) Elevated CO_2 concentration has independent effects on expansion rates and thickness of soybean leaves across light and nitrogen gradients. *Journal of Experimental Botany*, **49**, 583–591.

Sims DA, Seemann JR, Luo Y (1998b) The significance of differences in the mechanisms of photosynthetic acclimation to light, nitrogen and CO_2 for return on investment in leaves. *Functional Ecology*, **12**, 185–194.

Socias FX, Medrano H, Sharkey TD (1993) Feedback limitation of photosynthesis of *Phaseolus vulgaris* L. grown in elevated CO_2. *Plant, Cell and Environment*, **16**, 81–86.

Sokal RR, Rohlf FJ (1995) *Biometry. The principles and practice of statistics in biological research*. W. H. Freeman and Company, New York.

Staudt M, Joffre R, Rambal S, Kesselmeier J (2001) Effect of elevated CO_2 on monoterpene emission of young *Quercus ilex* trees and its relation to structural and ecophysiological parameters. *Tree Physiology*, **21**, 437–445.

Thornley JHM (1976) *Mathematical models in plant physiology. A quantitative approach to problems in plant and crop physiology*. Academic Press, London - New York - San Francisco.

Tognetti R, Johnson JD, Michelozzi M, Raschi A (1998) Response of foliar metabolism in mature trees of *Quercus pubescens* and *Quercus ilex* to long-term elevated CO_2. *Environmental and Experimental Botany*, **39**, 233–245.

Tosens T, Niinemets Ü, Vislap V, Eichelmann H, Castro-Díez P (2012) Developmental changes in mesophyll diffusion conductance and photosynthetic capacity under different light and water availabilities in *Populus tremula*: how structure constrains function. *Plant, Cell and Environment*, **35**, 839–856.

Trowbridge AM, Asensio D, Eller ASD *et al.* (2012) Contribution of various carbon sources toward isoprene biosynthesis in poplar leaves mediated by altered atmospheric CO_2 concentrations. *PLoS ONE*, **7**, e32387.

Velikova V, Tsonev T, Barta C *et al.* (2009) BVOC emissions, photosynthetic characteristics and changes in chloroplast ultrastructure of *Platanus orientalis* L. exposed to elevated CO_2 and high temperature. *Environmental Pollution*, **157**, 2629–2637.

Wilkinson MJ, Monson RK, Trahan N *et al.* (2009) Leaf isoprene emission rate as a function of atmospheric CO_2 concentration. *Global Change Biology*, **15**, 1189–1200.

Wolfertz M, Sharkey TD, Boland W, Kuehnemann F, Yeh S, Weise SE (2003) Biochemical regulation of isoprene emission. *Plant, Cell and Environment*, **26**, 1357–1364.

Yang RC, Kozak A, Smith JHG (1978) The potential of Weibull-type functions as flexible growth curves. *Canadian Journal of Forest Research*, **8**, 424–431.

Zimmer W, Brüggemann N, Emeis S, Giersch C, Lehning A, Steinbrecher R, Schnitzler JP (2000) Process-based modelling of isoprene emission by oak leaves. *Plant, Cell and Environment*, **23**, 585–595.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Illustration of a representative light-dark transient for *in vivo* estimation of dimethylallyldiphosphate (DMADP) pool size, and determinations of isoprene synthase rate constant.

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III

Elevated growth [CO_2] enhances isoprene emissions under high temperatures and improves thermal resistance in hybrid aspen

Zhihong Sun, Katja Hüve, Vivian Vislap and Ülo Niinemets

[1]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51014, Estonia

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Abstract

Isoprene emission is an important trait protecting plants from heat stress, but isoprene emission is inhibited by instantaneous increase of [CO_2], and it is currently unclear how isoprene-emitting plants cope with future more frequent and severe heat episodes under high [CO_2]. Hybrid aspen (*Populus tremula x P. tremuloides*) saplings grown under ambient [CO_2] of 380 μmol mol^{-1} and elevated [CO_2] of 780 μmol mol^{-1} were used to test the hypothesis that elevated [CO_2]-acclimated plants have greater stress resistance by enhanced isoprene emission rate, and to gain insight into the possible mechanism of regulation of isoprene emissions during the thermal stress. Elevated-[CO_2]-grown plants had higher cells membrane phase-transition temperature. When leaf temperature was enhanced from 30 ^oC to 50 ^oC, net assimilation rate decreased while isoprene emission rate increased for both growth [CO_2] treatments. However, plants grown under elevated [CO_2] had a lower depression of net assimilation rate coupled with higher isoprene emission rate, in particular when measured under strong light. There was a linear relationship between the reduction of net assimilation rate and increase of isoprene rate, but the slope was higher in plants grown under ambient [CO_2] than in plants grown under elevated [CO_2]. The inhibition of isoprene emission by increases in measurement [CO_2] disappeared when temperature was enhanced, and isoprene emission rate was mainly related to plant growth [CO_2]. These data indicated greater heat stress resistance in elevated-[CO_2]-grown plants that may reflect enhanced isoprene formation during stress. We suggest that greater isoprene emission is associated with higher "old" carbon pools that can be mobilized for isoprene formation upon heat stress.

Keywords: CO_2 response, elevated [CO_2], foliage traits, isoprene emission, heat stress, regulation of isoprene formation, temperature response

Introduction

Continuous rise of atmospheric CO_2 concentration is an important issue in global climate change (Ainsworth *et al.*, 2005, Luo, 2007). Atmospheric CO_2 concentrations have increased from 280 μmol mol^{-1} in preindustrial times to the current level of approximately 395 μmol mol^{-1} and [CO_2] is predicted to continue to rise to as much as 700–1000 μmol mol^{-1} by the year 2100 (de Graaff *et al.*, 2006, Luo, 2007). CO_2 elevation can enhance carbon uptake and net primary production by speeding up photosynthesis (Ainsworth *et al.*, 2008, Devi *et al.*, 2008, Qian *et al.*, 2010), but it can also alter plant leaf or fruit chemical composition such as carbon and nitrogen content and their ratio (Kant *et al.*, 2012, Tripp *et al.*, 1991). Furthermore, the rise of global atmospheric CO_2 concentration is associated with global warming; global surface temperatures are expected to increased by 1.1-6.4 ^oC by the end of this century if the consumption of fossil fuels stays at the present level (Luo, 2007). Thus, it is very likely that plants in future environments exposed to elevated [CO_2] also suffer from more severe heat and drought stresses.

Isoprene is the most abundant reactive volatile hydrocarbon emitted from a wide range of plant species (Fineschi *et al.*, 2013, Sharkey *et al.*, 2012, Monson *et al.*, 2012b). As a highly reactive volatile, isoprene significantly influences air quality by participating in ozone-forming reactions, and can also influence climate by participating in secondary organic aerosol formation(Claeys *et al.*, 2004). Isoprene as a small liphophilic molecule further plays important biological roles in protecting plants from abiotic stresses. Three hypotheses have been postulated to explain how isoprene protects from abiotic stresses, including 1) dissipation of excess energy to avoid photoinhibition (Niinemets *et al.*, 1999b, Sanadze, 2004); 2) thermoprotection by stabilizing biomembranes at high temperatures (Owen *et al.*, 2005, Sharkey *et al.*, 2001b, Singsaas *et al.*, 1997, Siwko *et al.*, 2007); 3) quenching reactive oxygen species formed under stress (Affek *et al.*, 2002, Loreto *et al.*, 2001, Vickers *et al.*, 2009).

Some evidence of all three biological functions of isoprene has been found in many previous investigations using different methods and different plant species. It has been reported that leaves in which isoprene biosynthesis has been inhibited by fosmidomycin, were more sensitive to high temperature and ozone exposure, and developed stronger

oxidative damage, compared to non-inhibited leaves (Loreto *et al.*, 2001, Peñuelas *et al.*, 2005, Sharkey *et al.*, 2001b, Singsaas *et al.*, 2000). Studies fumigating isoprene-non-emitting plants with isoprene have found that leaves were less damaged under thermal and oxidative stresses, and recovered more rapidly from heat stress than untreated controls (Loreto *et al.*, 2001, Sharkey *et al.*, 2001b, Singsaas *et al.*, 1997, Velikova *et al.*, 2006). Photosynthesis was inhibited by strong light intensity and this was accompanied with heavy membrane damage and higher amount of reactive oxygen species (ROS) in isoprene-inhibited leaves compared with non-inhibited leaves (Velikova *et al.*, 2005). In transgenic lines engineered to emit isoprene, isoprene maintained the macro-organization of the pigment-protein complexes in the membranes and stabilized the light-induced transmembrane electric field and recombination of the PSII donor and acceptor side charges (Velikova *et al.*, 2011), overall improving foliage photosynthesis under heat (Behnke *et al.*, 2007, Behnke *et al.*, 2013).

In plants, isoprene is formed in plastids from its immediate precursor dimethylallyldiphosphate (DMADP) by isoprene synthase (for reviews see Sharkey *et al.*, 2012, Li *et al.*, 2013, Rosenkranz *et al.*, 2013), and the control of isoprene synthesis under different environmental conditions is shared between isoprene synthase and DMADP pool size (Li *et al.*, 2012, Li *et al.*, 2013, Rasulov *et al.*, 2009b, Rasulov *et al.*, 2010, Sun *et al.*, 2012b, Monson, 2013). Isoprene emissions increase hyperbolically with increasing light intensity and depend on temperature according to an asymmetric response curve with an optimum (Monson *et al.*, 2012a for a review). In addition, increases of CO_2 concentration above the current ambient level inhibit isoprene emission (Possell *et al.*, 2011, Wilkinson *et al.*, 2009a, Monson *et al.*, 2012a). Isoprene emission responses to environmental drivers have been simulated assuming independent controls by different environmental drivers (for recent reviews see Monson *et al.*, 2012a, Grote *et al.*, 2013), and thus, it has been suggested that isoprene emissions will decline in the future due to increases in atmospheric [CO_2] concentration (e.g., Arneth *et al.*, 2007, Heald *et al.*, 2009, Wilkinson *et al.*, 2009a). This would imply reduced capacity of plants to cope with recurrent heat episodes by isoprene emission in future conditions. So far, there is limited information of the CO_2-sensitivity of isoprene emissions under heat stress (Li *et al.*, 2013 for a review), but we argue that direct extrapolation based on additive dependencies is not warranted.

Furthermore, isoprene emissions can acclimate to growth [CO_2] concentration, resulting

in altered [CO_2]-sensitivity of isoprene emission as well as in changes in the emission capacity (Wilkinson *et al.*, 2009, Sun *et al.*, 2012b, Calfapietra *et al.*, 2007, Calfapietra *et al.*, 2008, Calfapietra *et al.*, 2013). In fact, there is little evidence of downregulation in isoprene emission capacity under elevated [CO_2], and the emission capacity may even increase in elevated-[CO_2]-acclimated plants (Sun *et al.*, 2012b, Sharkey *et al.*, 1991, Li *et al.*, 2009). Such an elevation of emission capacity may partly compensate for reduction of emissions due to limited DMADP pool size under high ambient [CO_2], especially under high light (Sun *et al.*, 2012b). However, the overall effect of [CO_2] acclimation on isoprene emissions under high temperatures will depend on temperature-dependent changes in [CO_2]-sensitivity of emissions.

Given the enhanced severity of heat stress in future climates, it is necessary to gain detailed insight into plant possible responses and adaptation to altered environments. In this study, we investigated how photosynthesis and isoprene emission rates in hybrid aspen (*Populus tremula* x *P. tremuloides*) grown under different CO_2 concentrations respond to heat stress. We hypothesized that plants grown under elevated CO_2 have greater heat tolerance of photosynthetic apparatus and sustain greater isoprene emission rates especially under supra-optimal temperatures. Plants grown under elevated CO_2 had higher photosynthesis and isoprene emission rates under thermal stress and isoprene emissions in these plants responded more sensitively to temperature. Furthermore, the inhibition of isoprene emission by increasing [CO_2] was abolished under thermal stress. These results provide important insight into acclimation of photosynthesis and isoprene emission to growth CO_2, and the role of isoprene in thermotolerance. These results can also importantly contribute to development of process-based models for future climates.

Material and methods

Plant material and growth system

For these experiments, two-year-old saplings of hybrid aspen (*Populus tremuloides* Michx. x *P. tremula* L.) clone H200 were used (Rasulov *et al.*, 2009a, Rasulov *et al.*, 2011, Vahala *et al.*, 2003 for details of the genotype). Before the start of the experimental treatments, the

saplings were kept in cold room at -2 ºC in dormant state. Dormant plants were planted in 3 L plastic pots filled with sand and peat mixture (1:1), and dormancy was broken by transferring the plants to growth room at 20 ºC for 4 d. Plants with enlarged buds were installed in the open whole-plant gas-exchange/growth system for different [CO_2] treatments. During plant growth, nutrient and water supply was maintained at close to optimal level (Sun *et al.*, 2012a, Sun *et al.*, 2012b for details of plant growth).

The four-chamber whole-plant open gas-exchange/growth system design and operation have been described in our earlier studies (Sun *et al.*, 2012a, Sun *et al.*, 2012b). Shortly, each individual glass chamber had 12.5 L volume (diameter 0.2 m, height 0.4 m) to accommodate the entire foliage of a sapling, and flow rate through the chamber was 7.5 L min^{-1}, resulting in a relatively low chamber half-time of 70 s (Niinemets, 2012 for a comparison of whole-plant gas-exchange systems). The chambers 1 and 3 were kept at the ambient CO_2 concentration (average ± SD) of 380 ± 10 μmol mol^{-1}, and chambers 2 and 4 were treated with the elevated CO_2 concentration of 780 ± 10 μmol mol^{-1}. Chamber air temperature was maintained at 28-30/23 ^{o}C (day/night) and relative humidity at 60%. Photoperiod length was 12 h and the light intensity on the top of the plants was 500 μmol m^{-2} s^{-1} at start of the experiment, increasing to 800 μmol m^{-2} s^{-1} by the end of the experiments when the plants had filled the growth chamber (Sun *et al.*, 2012a, Sun *et al.*, 2012b).

After 30-40 days growth under given conditions, plants were randomly moved out and isoprene emission measurements and temperature stress treatments were carried out in individual attached fully mature leaves. The experiment was replicated four times, altogether with 16 plants in two treatment CO_2 concentrations.

Measurements of temperature responses of photosynthesis and isoprene emission

A Walz GFS-3000 portable gas exchange/chlorophyll fluorescence system equipped with a LED-array/PAM-fluorometer 3055-FL (Walz GmbH, Effeltrich, Germany) and linked with a Fast Isoprene Sensor (FIS, Hills-Scientific, Boulder, CO, USA) was used for combined measurements of photosynthetic characteristics and isoprene emission rates as described in detail in Sun *et al.* (2012a). The measurements were started by clamping the leaf in the cuvette and establishing the baseline conditions of leaf temperature of 30 ^{o}C, light intensity of 500 μ mol m^{-2} s^{-1} and relative humidity of 60%, corresponding to the environmental

conditions during plant growth. Temperature responses of photosynthesis and isoprene emission were measured after steady-state conditions had been established in the baseline conditions at both growth light intensity of 500 μ mol m^{-2} s^{-1} and strong light intensity of 2000 μ mol m^{-2} s^{-1}, and at both CO_2 concentrations of 380 μ mol mol^{-1} and 780 μ mol mol^{-1} using separate leaves for each combination of light and [CO_2]. We denote the growth [CO_2] treatments (380 vs. 780 μmol mol^{-1}) as Ambient and Elevated, and measurement CO_2 concentrations (380 vs. 780 μmol mol^{-1}) as 380 and 780, yielding four combinations of growth and measurement CO_2 concentrations: Ambient (380), Ambient (780), Elevated (380) and Elevated (780).

During response curve measurements, leaf temperature was changed from the baseline temperature of 30 ^{o}C to higher temperatures in steps of 5 ^{o}C up to 50 ^{o}C. The leaf was maintained at every temperature for 8 min. that was sufficient for establishment of steady-state conditions for measurements under 35-45 ^{o}C. Time-dependent reduction in photosynthetic rate was observed at 50 ^{o}C and sometimes at 45 ^{o}C as reported in other studies (Hüve *et al.*, 2011, Hüve *et al.*, 2006), and standardizing the time of sampling allows for comparison of all plants at a common heat dose.

Net assimilation, transpiration, and isoprene emission rates and steady-state fluorescence yield, F, were recorded during the last 30 s measurement period at the given temperature. Thereafter, a saturating pulse of white light was given to measure the maximum light-adapted quantum yield of photosystem II (PSII), F_m'. The effective quantum yield of PSII (Φ_{PSII}) was determined as (F_m'-F)/F_m'.

Isoprene concentration in leaf intercellular air space ($C_{iso,i}$) was calculated as:

$$C_{iso,i} = C_{iso,a} + \frac{I}{g_{s,iso}} \quad (1)$$

where $C_{iso,a}$ is the isoprene concentration in the leaf chamber, and $g_{s,iso}$ is the stomatal conductance for isoprene. $g_{s,iso}$ was estimated as the product of stomatal conductance to water vapor and the ratio of the binary diffusion coefficients for isoprene (D_{iso}) and water vapor (D_{H2O}) (Niinemets *et al.*, 2003). The temperature relationships of D_{iso} and D_{H2O} were developed based on the Chapman and Enskog theory of gas diffusion by intermolecular collision as in Niinemets and Reichstein (2003). The ratio D_{iso}/D_{H2O} was essentially independent of temperature, and an average value of 0.339 was used.

Estimation of relative changes in assimilation and isoprene emission

To compare the temperature treatment effects independent of differences in the capacities for net assimilation and isoprene emission, we calculated the normalized change in net assimilation rate, R_A, as:

$$R_A = \frac{A_{30} - A_T}{A_{30}}, \quad (2)$$

where A_{30} is the net assimilation rate at 30 ºC and A_T that at given temperature T. An increase in R_A reflects a reduction in A_T compared with A_{30}. Relative change in the effective quantum yield of PSII was calculated analogously. Relative change in isoprene emission rate due to changes in temperature, R_I, was calculated as:

$$R_I = \frac{I_T - I_{30}}{I_{30}}, \quad (3)$$

where I_{30} is the isoprene emission rate at 30 ºC and I_T the emission rate at given temperature T. An increase in R_I corresponds to an increase in I_T relative to I_{30}. As net assimilation rate generally decreased and isoprene emission rate increased beyond 30 ºC, relative changes in assimilation and isoprene emission were defined differently to have positive values for both R_A and R_I across the whole temperature range.

Electrolyte leakage in response to heat stress

Leaf relative electrolyte leakage, a measure of membrane integrity, was assessed by changes in electrical conductivity of distilled water after soaking the treated leaves (Bajji *et al.*, 2002, Scotti Campos *et al.*, 2003, Kocheva *et al.*, 2005). Detached leaves enclosed in plastic bags were immersed in water at given temperature (25, 50 and 52 ºC) for 5 min. Then, three freshly cut discs (7 mm in diameter each) from the treated leaf were immediately soaked in 5 ml of distilled water at 25 ºC. Conductivity of the water was measured in 24 h after disc soaking using a conductometer HandyLab LF1 (Schott GmbH, Germany). Thereafter, the same flasks with leaf discs were heated in boiling water bath for 10 min. and let to cool for 1 h. The solution conductivity was measured again at 25 ºC, and the relative electrical conductivity of the sample was expressed as the percentage of maximum conductivity observed after boiling.

Foliage morphological and anatomical measurements

After gas exchange measurements, leaf samples were taken for structural and chemical analyses. Leaf fresh mass and leaf area were determined immediately and dry mass after drying the leaves in ventilated oven at 70 ºC for 48 h. Key foliage structural, anatomical and chemical traits, including leaf dry mass per unit area (M_A), nitrogen and carbon content, leaf thickness, exposed mesophyll and chloroplast surface area, and number of chloroplasts for leaves developed under the CO_2 treatments (10-16 replicates per treatment) have been reported in Sun et al. (2012, 2013). Elevated-[CO_2]-grown plants had ca. 15% thicker leaves with ca. 35% greater M_A and ca. 20% greater chloroplast exposed surface area per leaf area (Sun *et al.*, 2012b). In addition, the cross-sectional area of chloroplasts covered by starch granules per chloroplast area ($a_{chl,s}/a_{chl}$) was ca. 50% greater under elevated [CO_2] (Sun *et al.*, 2012b). Here we use these data to estimate the distribution of leaf water among different leaf fractions.

First, the volume fraction of mesophyll without intercellular air space ($f_{t,mes}$) was calculated as:

$$f_{t,mes} = \frac{t_{mes}}{t} - f_{ias}, \quad (4)$$

where t_{mes} is the mesophyll thickness and t the leaf thickness and f_{ias} is the fraction of intercellular air space. The volume fraction of chloroplasts ($f_{t,chl}$) was calculated as the product of $f_{t,mes}$ and the ratio of cross-sectional areas of chloroplasts to mesophyll cells (a_{chl}/a_{mes}). The fraction of water in leaf mesophyll, $F_{W,mes}$, was approximated by:

$$F_{W,mes} = \frac{f_{t,mes}}{1 - f_{t,cut}}, \quad (5)$$

where $f_{t,cut}$ is the volume fraction of cuticle with outer thickened cell walls (Niinemets, 1999). Equation 5 assumes that leaf water is uniformly distributed among epidermis and mesophyll cells. The correction, $f_{t,cut}$, was minor for hybrid aspen, but was included for the internal consistency. Finally, the volume fraction of water in chloroplasts, $F_{W,chl}$, was calculated as:

$$F_{W,chl} = f_{W,mes} \frac{a_{chl}}{a_{mes}} \left(1 - \frac{a_{chl,s}}{a_{chl}}\right). \quad (6)$$

The second term in this equation, $1-a_{chl,s}/a_{chl}$, accounts for the reduction of chloroplast water due to presence of starch granules.

Leaf sugar analysis

Soluble sugar content was measured with the phenol sulphuric acid method of Dubois et al. (1956) as modified by Chow and Landhäusser (2004). The method is based on formation of orange-red color as the result of condensation of furan derivatives produced under acidic conditions with phenol (Dubois *et al.*, 1956). The soluble sugars were extracted in distilled water at 100 ^{o}C for 30 min, the extract was treated with the phenol/sulphuric acid reagent as in Chow and Landhäusser (2004) and the absorbance was measured at 485 nm with a Shimadzu UV2550PC spectrophotometer (Shimadzu, Kyoto, Japan). The standard curve was developed for sucrose and finally the sugar content was expressed in C_6 sugar units. Leaf sugar concentration was calculated both per unit leaf dry mass and per unit leaf water.

Data analyses

The [CO_2] treatment effects on leaf traits were compared by one-way ANOVA followed by Tukey's test. Within treatments, paired samples *t*-tests were used to compare the physiological traits measured repeatedly under different measurement light and [CO_2] conditions.

Correlative relationships of R_A (Eq. 2) vs. R_I (Eq. 3) were analyzed by linear regressions, and whenever pertinent by second order polynomial regressions. Co-variation analyses (ANCOVA) were employed to compare these relationships among the [CO_2] treatments and at different measurement [CO_2] and light intensities. Whenever necessary in R_A and R_I vs. temperature responses, second order temperature term, T x T, and its interaction with the main effect(s) was included in the model to account for curvilinear effects. In the analysis, the significance of interaction terms was tested first (separate slope model) and whenever the interaction terms was non-significant, the model was refitted without the interaction term (common slope model). SPSS 17.0 (IBM SPSS Statistics) was used for all analyses, and all statistical relationships were considered significant at $P < 0.05$.

Results

Effects of growth [CO_2] on leaf structure and chemistry

As we have demonstrated previously in hybrid aspen (*Populus tremula x P. tremuloides*),

elevated [CO_2] resulted in thicker leaves with greater leaf dry mass per unit area and more chloroplasts per unit leaf surface area, overall indicating more advanced mesophyll development (Sun *et al.*, 2012b, Sun *et al.*, 2013). Here we analyze additional traits with importance in leaf heat tolerance. Elevated growth [CO_2] resulted in greater leaf fresh mass per unit leaf area (M_F) and mass of water per leaf area (M_{WA}), though there was no significant treatment effect on mass of water per leaf volume (M_{WV}) (Table 1). The fractions of mesophyll cells and intercellular airspace of total leaf volume did not differ among the treatments, but the volume percentage of chloroplasts was higher in elevated-[CO_2]-grown leaves (Table 1). Although starch granules comprised a greater proportion of chloroplast volume in leaves under elevated [CO_2] treatment (Fig. 1), the overall fraction of leaf water in chloroplasts (Eq. 6) was higher under elevated [CO_2] (Table 1). Leaf soluble sugar contents per dry mass (S_D) and per leaf water (S_W) were greater in leaves under elevated [CO_2] (Table 1).

Dependence of photosynthesis and isoprene emission rate and intercellular isoprene concentration on temperature: general patterns

Net assimilation rate (A) of hybrid aspen leaves was the highest at leaf temperatures between 30-35 ºC (Fig. 2a, b), while isoprene emission rate (I) increased up to temperatures of 45-50 ºC (Fig. 2c, d). Temperature responses were broadly similar under moderately high light intensity of 500 μmol m^{-2} s^{-1} and strong light intensity of 2000 μmol m^{-2} s^{-1} (cf. Figs. 2a and 2b and Figs. 2c and 2d).

The temperature dependence of the concentration of isoprene in leaf intercellular air space ($C_{iso,i}$) mirrored the temperature response of isoprene emission (Fig. 3a, b), while the fraction of carbon lost due to isoprene emission was the highest at 50 ºC, reaching up to 15% of photosynthesis rate, i.e. almost an order of magnitude increase compared to the carbon loss at 30 ºC (Fig. 3c, d).

Effects of growth [CO_2] and measurement [CO_2] and light intensity on net assimilation and isoprene emission rates under different T

Measurement CO_2 generally increased the net assimilation rates (Fig. 2a, b), although the increase was weaker for plants grown under ambient [CO_2] than in plants grown under

elevated [CO_2], especially under moderately high light intensity of 500 μmol m^{-2} s^{-1} (cf. Figs. 2a and 2b). At light intensity of 500 μmol m^{-2} s^{-1} and measurement [CO_2] of 380 μmol mol^{-1}, *A* was similar among plants grown at ambient and elevated [CO_2] (Fig. 2a), but when measured at [CO_2] of 780 μmol mol^{-1}, *A* of elevated-[CO_2]-grown plants was higher than in ambient-[CO_2]-grown plants at given temperature (Fig. 2a). Furthermore, under the strong light intensity of 2000 μmol m^{-2} s^{-1}, *A* in elevated-[CO_2]-grown plants was significantly higher than that in ambient-[CO_2]-grown plants at both measurement CO_2 concentrations of 380 μmol mol^{-1} and 780 μmol mol^{-1} (Fig. 2b).

Higher measurement [CO_2] inhibited isoprene emission rate in elevated-[CO_2]-grown plants at temperatures 30-35 ºC under moderately high light (Fig. 2c) and at 30 ºC under strong light (Fig. 2d), but the [CO_2]-inhibition was lost at higher temperatures (Fig. 2c, d). At temperatures beyond 35 ºC under moderately high light and beyond 30 ºC under strong light, isoprene emission rate in elevated-[CO_2]-grown plants exceeded that in ambient-[CO_2]-grown plants (Fig. 2c, d).

The variations in $C_{iso,i}$ among the growth [CO_2] treatments and measurement [CO_2] and light intensities reflected the differences in isoprene emission rate (cf. Fig. 2c, d and Fig. 3a, b). Thus, $C_{iso,i}$ was greater at stronger light, did not depend on measurement [CO_2], and was greater in elevated-[CO_2]-grown plants beyond 35 ºC under the moderately high light intensity of 500 μmol m^{-2} s^{-1} and beyond 30 ºC under the strong light intensity of 2000 μmol m^{-2} s^{-1} (Fig. 3a, b).

The fraction of carbon lost as isoprene was always larger at the measurement [CO_2] of 380 μmol mol^{-1} than at 780 μmol mol^{-1} (Fig. 3c, d). Growth [CO_2] effects on relative carbon loss were minor with the only significant difference being the greater carbon loss at 30 ºC under moderately high light intensity and at measurement [CO_2] of 380 μmol mol^{-1} in ambient-[CO_2]-grown plants (Fig. 3c).

Relationships of decreased photosynthesis with increased isoprene emission rate through the temperature response

The relative decrease of net assimilation rate with increasing temperature (Eq. 2, R_A) was almost linearly related to temperature between temperatures 35-50 ºC (Fig. 4a, b), while the temperature response of the relative increase of isoprene emission rate (Eq. 3, R_I) was

curvilinear, saturating at temperatures 45-50 ºC (Fig. 4c, d, the second order effect, *T* x *T*, was significant in all cases, $P < 0.001$).

According to the co-variation analyses, the interaction terms, *T* x (measurement [CO_2]) and *T* x (growth [CO_2]) were generally significant ($P < 0.05$, except for Fig. 4a), indicating a smaller variation in R_A and R_I at lower temperatures. At higher temperatures of 40-50 ºC, R_A was significantly less at given temperature for elevated-[CO_2]-grown plants measured at [CO_2] of 780 μmol mol^{-1} ($P < 0.02$ for the response measured at light intensity of 500 μmol m^{-2} s^{-1} for all temperature range and $P < 0.001$ at 2000 μmol m^{-2} s^{-1}; Fig. 4a, b). For the temperature range 40-50 ºC, R_I was the greatest for elevated-[CO_2]-grown plants measured at [CO_2] of 780 μmol mol^{-1} and the smallest for ambient -[CO_2]-grown plants measured at [CO_2] of 380 μmol mol^{-1} ($P < 0.001$), while the two other combinations (elevated-grown measured at 380 μmol mol^{-1} and ambient-grown measured at 780 μmol mol^{-1}) did not differ ($P > 0.7$, Fig. 4c, d). Reductions in the effective quantum yield of PSII paralleled changes in R_A, being smaller in elevated-[CO_2]-grown plants measured at [CO_2] of 780 μmol mol^{-1} than in the other treatments ($P < 0.001$). The reductions in PSII quantum yield and in R_A were correlated across the different measurement conditions and treatments (Fig. 5).

The reduction of net assimilation rate was positively correlated with the increase in isoprene emission rate (Fig. 6a, b). For measurements under lower light intensity of 500 μmol m^{-2} s^{-1}, the interaction of R_I with treatment was not significant ($P > 0.8$). According to the common slope ANCOVA, ambient-[CO_2]-grown leaves measured at 380 μmol mol^{-1} had greater R_A at given R_I than the other treatments, while elevated-CO_2-grown leaves measured at 780 μmol mol^{-1} had lower R_A at given R_I than the other treatments (Fig. 6a, $P < 0.001$ for both comparisons including all the replicate measurements). At higher light of 2000 μmol m^{-2} s^{-1}, the interaction term was significant, indicating that the slope for elevated-[CO_2]-grown plants measured at 780 μmol mol^{-1} was less than for the other growth and measurement [CO_2] combinations (Fig. 6b, $P < 0.001$ for the analysis including all the replicate measurements).

Membrane leakiness in relation to growth [CO_2] and temperature-dependent reduction in net assimilation rate

Relative electrical conductivity, the measure of membrane leakiness, was not significantly increased in elevated-[CO_2]-grown plants after exposure of leaf discs to 50 ºC (Fig. 7a). In

contrast, in ambient-[CO_2]-grown plants, exposure to 50 ºC resulted in a significant increase in membrane leakiness (Fig. 7a). Exposure to severe heat stress of 52 ºC resulted in enhanced membrane leakiness for both [CO_2] treatments, but the leakiness was greater for ambient-[CO_2]-grown plants (Fig. 7a).

Relative electrical conductivity at 50 ºC was correlated with the reduction in net assimilation rate (Fig. 7b) and PSII quantum yield (data not shown). However, the slope of this relationship was shallower in ambient-[CO_2]-grown plants ($P < 0.001$ for the interaction term of electrical conductivity x (growth [CO_2], Fig. 7b), indicating that in ambient-[CO_2]-grown plants, a given reduction in net assimilation rate observed immediately at the end of the exposure period was associated with greater electrolyte leakage over the following 24 hr soaking of leaf disks.

Discussion

Elevated [CO_2]-driven modifications in leaf chemistry, structure and photosynthesis

Elevated growth [CO_2] resulted in greater leaf fresh mass per unit leaf area (M_F), mass of water per leaf area (M_{WA}) and fraction of water in chloroplasts ($F_{W,Chl}$) (Table 1). However, the mass of leaf water per leaf volume was not significantly different among the treatments, indicating that greater mass of water per leaf area resulted from thicker leaf mesophyll in elevated-[CO_2]-grown plants (Sun *et al.*, 2012b) as has been consistently observed (e.g., Sims *et al.*, 1998b, Sims *et al.*, 1998a, Miyazawa *et al.*, 2011), and suggested to reflect morphological "upregulation" (Luo *et al.*, 1997).

Elevated [CO_2] also resulted in greater starch grain number and size inside the chloroplasts (Fig. 1) and enhanced leaf sugar content per dry mass and concentration in leaf water (Table 1) as has been demonstrated in numerous studies (Saxe *et al.*, 1998, Makino *et al.*, 1999 for reviews). Typically, elevated [CO_2] is associated with "downregulation of photosynthesis" defined as reduced photosynthesis observed at the same given ambient [CO_2] (Nowak *et al.*, 2004, Luo *et al.*, 1997, Curtis *et al.*, 1998, Johnson, 2006). This down-regulation is mainly associated with reduced nitrogen content and may also reflect feedback-inhibition of photosynthesis due to enhanced sugar concentrations (Myers *et al.*, 1999, Jeannette *et al.*, 2000, Nowak *et al.*, 2004, Luo *et al.*, 1997, Curtis *et al.*, 1998, Johnson,

2006). However, in our study at optimum nutrient supply, we actually observed enhanced photosynthetic capacity in elevated-[CO_2]-grown plants (Sun *et al.*, 2012b, Fig. 2b), indicating no downregulation or stronger feedback inhibition despite of higher sugar concentrations.

Temperature responses of net assimilation and isoprene emission under different environmental conditions

Isoprene is formed in chloroplasts by isoprene synthase from its immediate precursor dimethylallyldiphosphate (DMADP, Li *et al.*, 2013 for a recent review). The major source of chloroplastic DMADP is the plastidic 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway that starts with condensation of pyruvate (Pyr) and glyceraldehyde-3-phosphate (GAP) (Lichtenthaler, 1999, Schwender *et al.*, 1997).

As isoprene synthase and the main pathway for DMADP formation are in chloroplasts, and chloroplastic DMADP formation strongly relies on recently fixed carbon, in particular, on primary photosynthetic metabolite GAP, isoprene emission is strongly associated with photosynthetic carbon metabolism in non-stressed conditions (Monson, 2013, Li *et al.*, 2013 for recent reviews).

However, there are important discrepancies among isoprene emission and photosynthesis as demonstrated by our study and past observations, indicating significant differences in the regulation of isoprene emission and net assimilation rate: 1) the optimum temperature for isoprene emission is larger than that for net photosynthesis (Fig. 2 and e.g., Niinemets *et al.*, 1999b, Harley *et al.*, 1996) and as the result, the fraction of carbon lost due to isoprene emission increases at higher temperatures (Fig. 3c, d); 2) isoprene emission more strongly responds to light than net assimilation rate and is saturated at greater light intensity (Fig. 2-3 and e.g., Niinemets *et al.*, 1999b, Harley *et al.*, 1996, Niinemets *et al.*, 2010, Monson *et al.*, 2012a); 3) isoprene emission is inhibited by above-ambient [CO_2] concentrations, while photosynthesis increases (Fig. 2, Li *et al.*, 2013, Monson, 2013 for reviews). Apart from these general observations, our study demonstrates a number of important novel aspects of environmental responses of isoprene emission under high temperature and in plants developed in different [CO_2]: 1) the CO_2-sensitivity of isoprene emission was lost at temperatures beyond 35-40 ºC (Fig. 2c, d); 2) as the result of the loss of

[CO_2]-sensitivity of emissions, isoprene emission rates in elevated-[CO_2]-grown plants exceeded the emissions in ambient-[CO_2]-grown plants at higher temperatures of 40-50 ºC not only at high but also at moderate light intensity (Fig. 2c, d; Fig. 4c, d);

These results of our study highlight a complex interplay between different environmental drivers and growth [CO_2] treatments on leaf isoprene emission. There is evidence that both instantaneous light- and [CO_2]-dependencies of isoprene emission are primarily driven by light- and [CO_2]-driven changes in DMADP pool size (Rasulov *et al.*, 2009a, Rasulov *et al.*, 2009b, Li *et al.*, 2011, Li *et al.*, 2012, Possell *et al.*, 2011), while the temperature dependence is a mixed response, driven both by temperature-dependent changes in DMADP pool size and isoprene synthase activity (Rasulov *et al.*, 2010, Rasulov *et al.*, 2011, Li *et al.*, 2011, Li *et al.*, 2012). As we have demonstrated in our previous study (Sun *et al.*, 2012b), elevated-[CO_2]-grown plants had greater isoprene synthase activity, but lower DMADP pool size (Sun *et al.*, 2012b). In the following, we address the facets of the isoprene emission response to these complex multifactorial environmental interactions and acclimation responses based on the immediate effects of environmental conditions on the rate of DMADP synthesis as well as growth-[CO_2]-dependent changes in overall DMADP pool size and isoprene synthase activity.

Carbon sources for isoprene formation under heat stress

It has been reported that in non-stressed plants, 84–88% of the carbon in released isoprene was derived from recently assimilated photosynthates, while the contribution of recent photosynthates to isoprene production decreased to 77 and 61% in heat and water-stressed leaves, respectively (Funk *et al.*, 2004). Similarly, it has been reported that in *Populus alba*, isoprene emission rate was about 30% of the pre-stress value even during the most severe phase of stress, when photosynthesis was totally inhibited (Brilli *et al.*, 2007). This evidence indicates that “old” stored carbon sources can be mobilized for isoprene formation under stress conditions when photosynthesis rates decline.

Under thermal stress, photosynthesis is decreased and this is further coupled to large amounts of ROS produced (Hüve *et al.*, 2011). Thus, large amounts of isoprene might be needed for ROS quenching, and it is likely that isoprene formation will mobilize all available carbon sources. We suppose that there are three ways of how to enhance carbon flow to

isoprene formation under stress. First is the mobilization of old or temporary stored photosynthates like starch. Glycolysis and in particular pentose phosphate (PPP) pathway in stressed plants are the possible routes for the use of stored photosynthates (Eicks *et al.*, 2002, Fettke *et al.*, 2011). Under stress, enhancement of cyclic or pseudocyclic electron transport and reduction of the linear electron transport activity can result in imbalances of photophosphorylation and NADPH synthesis. PPP pathway can generate sugar phosphates (GAP) and large amounts of NADPH for the DOXP/MEP pathway, compensating for shortage of carbon and reductive equivalents from photosynthesis under stress. In addition, PEP and pyruvate can be produced by glycolysis and enter in the DOXP/MEP pathway (Rasulov *et al.*, 2011).

The second possible regulation point is isopentenyldiphosphate (IDP), the isomer of DMADP, flow between chloroplasts and cytosol. In the cytosol, DMADP and IDP are formed via mevalonic acid (MVA) pathway, and IDP may be transported between the cytosol and plastid. Many experiments have confirmed that there is a crosstalk between chloroplasts and cytosol (Laule *et al.*, 2003, Lichtenthaler, 2007), but MVA pathway contribution in non-stressed conditions is generally minor (Bick *et al.*, 2003, Laule *et al.*, 2003). However, the contribution might increase at higher temperatures, but so far, there is no experimental confirmation of temperature-dependent increases of cytosolic IDP contribution to isoprene synthesis.

The third regulation point is the change in the distribution of flows of DMADP into isoprene formation and into formation of other polyterpenes synthesized in chloroplasts. Under heat stress, protection of photosynthetic apparatus can be achieved via isoprene or polyterpenes such as xanthophylls. Because the carbon cost of isoprene is much lower compared with xanthophylls, allocating DMADP preferably to isoprene synthesis may provide a greater protection during recurrent heat stress episodes. This could be achieved by inhibiting geranyldiphosphate synthase or any other key prenyltransferase downstream of DMADP. So far, the information of regulation of prenyltransferase activities is limited, but clearly such a possible stress-dependent regulation deserves further experimental investigations. In the study of Peñuelas et al. (2005), α-tocopherol and β-carotene contents decreased after enhancement of isoprene emission rate during heat stress. This indirectly supports the suggestion that isoprene formation may have priority over synthesis of

polyterpenoids during heat stress.

Effects of elevated [CO_2] on photosynthesis under heat stress

In this study, elevated growth [CO_2] increased membrane stability under heat stress in hybrid aspen. Elevated-[CO_2]-grown plants maintained low electrolyte leakage at heat stress as severe as 50 ^{o}C, but this temperature resulted in major increases in membrane leakiness in ambient-[CO_2]-grown plants (Fig. 7a). Our data further indicated that elevated growth [CO_2] enhanced plant net assimilation rate, particularly at high measurement [CO_2] and higher light intensity. Previous studies have demonstrated that photosynthesis is particularly sensitive to heat stress. Already moderate heat stress reduces net assimilation rate (Essemine *et al.*, 2011, Sharkey, 2005), mainly leading to inactivation of photosynthetic electron transport, in particular PSII activity (Law *et al.*, 1999, Toth *et al.*, 2011). Exposure of plants to elevated temperatures results in inactivation of Rubisco activase (Barta *et al.*, 2010, Toth *et al.*, 2011) and the oxygen-evolving complex (OEC) of PSII, including the removal of extrinsic (surface) proteins as well as release of calcium and manganese ions from their binding sites (Barta *et al.*, 2010, Luo *et al.*, 2011). Heat stress also changes the structure of the thylakoid membranes by changing specific lipid–protein interactions for transmembrane proteins and dynamic properties of the lipid bilayer (Fristedt *et al.*, 2009, Pfeiffer *et al.*, 2005, Yin *et al.*, 2010, Yoshioka *et al.*, 2006). The thermal damage of photosynthetic apparatus leads to reduction of linear electron transport from PSII to PSI and decreased rate of photophosphorylation (Hald *et al.*, 2008). Inactivated OEC further leads to accumulation of highly oxidizing radicals, like singlet oxygen, hydroxyl radicals and H_2O_2 (Larkindale *et al.*, 2005, Suzuki *et al.*, 2006), which leads to a rapid inactivation and degradation of PSII reaction centers (Hald *et al.*, 2008, Haldimann *et al.*, 2004, Toth *et al.*, 2011, Zhu *et al.*, 2007). In this study, there was a strong linear correlation between the reductions in PSII quantum yield and net assimilation rate (Fig. 5), in agreement with the results of the past studies.

In this study, we observed that elevated-[CO_2]-grown plants had greater net photosynthesis rate at given temperature than those grown under ambient [CO_2] at both measurement [CO_2] of 380 μ mol mol^{-1} and 780 μ mol mol^{-1}, indicating that elevated-[CO_2]-grown plants had higher thermal tolerance of photosynthesis. The higher thermal tolerance of elevated-[CO_2]-grown plants was more notable under high light,

particular when measured at [CO_2] of 780 μ mol mol^{-1} (Fig. 4b; Fig. 6b). These results confirm the previous observations of greater heat tolerance of photosynthesis in elevated-[CO_2]-grown plants (Ainsworth *et al.*, 2005, de Graaff *et al.*, 2006, Sanz-Saez *et al.*, 2010, Taub *et al.*, 2000). In the following, we ask how acclimation to elevated growth [CO_2] and instantaneous [CO_2] increase can enhance heat tolerance of photosynthesis.

The possible mechanism of protection against heat damage of photosynthesis by growth and measurement [CO_2]

We suppose that there are several mechanisms contributing to greater heat tolerance of photosynthesis under high [CO_2]. First, elevated [CO_2] increases intercellular [CO_2], resulting in greater turnover of Rubisco at higher temperature and light, and greater consumption of excitation energy by photosynthetic electron transport, reducing excitation pressure to PSII (e.g., Niinemets *et al.*, 1999a). Plants have developed a repair system of PSII by continuous degradation and synthesis of the D1 protein in the PSII reaction centre, thereby preventing PSII photoinhibition even under strong light intensity (Yoshioka *et al.*, 2006). However, limited supply of CO_2 can reduce the rate of PSII repair (Tcherkez *et al.*, 2006).

Elevated [CO_2] also reduces photorespiration, thereby potentially decreasing the production of reactive oxygen species (ROS), especially formation of singlet oxygen. ROS inhibits the repair cycle of PSII by inhibiting protein synthesis in the chloroplast (Nishiyama *et al.*, 2006). This suggestion is in agreement with the finding that high intercellular [CO_2] stimulated photosynthetic electron transport and higher non-photochemical fluorescence quenching (Taub *et al.*, 2000).

The enhancement of thermal tolerance under elevated growth [CO_2] has also been associated with greater sugar concentrations that stabilize membranes under stress (Nagao *et al.*, 2005, Livingston D. *et al.*, 2009). In our study, elevated growth [CO_2] resulted in greater leaf sugar content in leaf water (Table 1). However, the overall effect of sugars on heat tolerance may depend on the subcellular distribution of sugars. Given the distribution of water within the leaf, greater proportion of sugars was associated in chloroplasts under elevated [CO_2], and thus, sugar concentrations were likely elevated both in the cytosol and chloroplasts in elevated-[CO_2]-grown plants. Thus, higher sugar concentrations seemed to contribute to the higher thermal tolerance by maintaining lower membrane leakage of hybrid

aspen plants grown under elevated [CO_2].

Do higher isoprene emission rates protect photosystem from injury by heat and strong light?

These data indicated that under thermal stress, leaf isoprene production was uncoupled from net assimilation rate, which is consistent with previous investigations indicating that isoprene emission rate became unrelated from photosynthesis rate under stress (Calfapietra *et al.*, 2008, Velikova *et al.*, 2005). In fact, isoprene emission was likely more dependent on stored carbon and growth [CO_2] concentration. As discussed above, heat stress leads to reduction of photosynthesis as the result of multiple processes including Rubisco deactivation, thylakoid membrane leakage, imbalanced electron transport between the two photosystems PSI and PS II and production of reactive oxygen species. In past decades, more and more experimental evidence has accumulated showing that isoprene can reduce the damage of photosynthetic apparatus under thermal and oxidative stress (Loreto *et al.*, 2010, Vickers *et al.*, 2009), though plant costs for isoprene emission can be very high (Niinemets *et al.*, 1999c, Sharkey *et al.*, 2001a). The protective function of isoprene has been attributed to: (1) stabilization and protection of membranes against high temperature (Sharkey *et al.*, 2001a, Singsaas *et al.*, 1997, Velikova *et al.*, 2011); (2) its antioxidant properties eliminating ROS produced by heat stress, and (3) consumption of excess energy (Sanadze, 2004, Sanadze, 2010). As discussed above, heat stress impacts photosynthesis in multiple ways, and isoprene as a highly reactive and small volatile has great potential to protect simultaneously from several adverse effects of heat stress.

Under heat stress, net assimilation rate was affected by both long-term growth [CO_2] and instantaneous measurement [CO_2] (Fig. 2a, b), while isoprene emission rate was affected mainly by its growth [CO_2], and elevated-[CO_2]-grown plants had significantly higher isoprene emission rate (Fig. 2c, d). Why did increased measurement [CO_2] depress isoprene emission rate under moderate temperature, while this inhibition disappeared under heat? In our previous study, we found that under moderate temperature of 30 ºC, the increase of measurement [CO_2] depressed isoprene emission rate due to decreased DMADP pool size. However, increase in growth [CO_2] decreased DMADP pool size and enhanced isoprene synthase activity (Sun et al 2012). At given isoprene synthase activity, the emission rate is

mainly regulated by its precursor DMADP pool size (Cinege *et al.*, 2009, Lehning *et al.*, 1999, Rasulov *et al.*, 2009a, Vickers *et al.*, 2010). However, temperature also affects isoprene synthase activity (Rasulov *et al.*, 2010) that can partly compensate for reduced DMADP pool size. Nevertheless, increases in temperature also should have enhanced DMADP synthesis, especially in elevated-[CO_2]-grown plants. As photosynthesis rate decreased, this increase likely reflected mobilization of stored carbon pools, a process that essentially occurs in [CO_2]-independent manner. As demonstrated in Table 1 and Fig. 1, elevated growth [CO_2] resulted in higher starch and soluble sugar content, possibly explaining greater enhancement of isoprene emission under heat stress in elevated-[CO_2]-grown plants.

Conclusions

The evidence of maintenance of enhanced isoprene emission capacity in elevated-[CO_2]-grown plants through moderately elevated temperature to temperatures resulting in severe heat stress as well as loss of CO_2-inhibition of isoprene emission at higher temperatures (Fig. 2c, d, Li *et al.*, 2011, Li *et al.*, 2013, Rasulov *et al.*, 2010) has major implications for predicting isoprene emissions in future climates. Apart from these key findings, our study further highlights a number of important differences in overall emission rates and among temperature responses under different growth [CO_2] treatments and under different measurement [CO_2] and light intensities that collectively suggest that the effects of environmental drivers interactively affect isoprene emission at the level of dimethylallyldiphosphate (DMADP) pool size. Thus, future models should focus on predicting integrated environmental controls on DMADP pool size rather than considering each environmental driver independently of others.

The results of this study further demonstrate that heat resistance of hybrid aspen was strongly enhanced by elevated growth-[CO_2] and this was associated both with more stable net assimilation rates as well as with particularly strong enhancement of isoprene emissions under heat stress. Thus, contrary to past suggestions, our results indicate that isoprene may protect leaf photosynthetic function under extreme heat waves more effectively under future elevated [CO_2] conditions. For understanding climate change effects on vegetation, it is important to consider impacts of acclimation to growth [CO_2] on foliage heat stress resistance.

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References

Affek HP, Yakir D (2002) Protection by isoprene against singlet oxygen in leaves. *Plant Physiology,* **129,** 269-277.

Ainsworth EA, Leakey ADB, Ort DR, Long SP (2008) FACE-ing the facts: inconsistencies and interdependence among field, chamber and modeling studies of elevated CO_2 impacts on crop yield and food supply. *New Phytologist,* **179,** 5-9.

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. *New Phytologist,* **165,** 351-371.

Arneth A, Niinemets Ü, Pressley S*, et al.* (2007) Process-based estimates of terrestrial ecosystem isoprene emissions: incorporating the effects of a direct CO_2-isoprene interaction. *Atmospheric Chemistry and Physics,* **7,** 31-53.

Bajji M, Kinet J-M, Lutts S (2002) The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regulation,* **36,** 61-70.

Barta C, Dunkle AM, Wachter RM, Salvucci ME (2010) Structural changes associated with the acute thermal instability of Rubisco activase. *Archives of Biochemistry and Biophysics,* **499,** 17-25.

Behnke K, Ehlting B, Teuber M*, et al.* (2007) Transgenic, non-isoprene emitting poplars don't like it hot. *The Plant Journal,* **51,** 485-499.

Behnke K, Ghirardo A, Esperschütz J*, et al.* (2013) Isoprene function in two contrasting poplars under salt and sunflecks. *Tree Physiology,* **xx,** In Press.

Bick JA, Lange BM (2003) Metabolic cross talk between cytosolic and plastidial pathways of isoprenoid biosynthesis: unidirectional transport of intermediates across the chloroplast envelope membranes. *Archives of Biochemistry and Biophysics,* **415,** 146-154.

Brilli F, Barta C, Fortunati A, Lerdau M, Loreto F, Centritto M (2007) Response of isoprene emission and carbon metabolism to drought in white poplar (Populus alba) saplings. *New Phytologist,* **175,** 244-254.

Brilli F, Tsonev T, Mahmood T, Velikova V, Loreto F, Centritto M (2013) Ultradian variation of isoprene emission, photosynthesis, mesophyll conductance, and optimum temperature sensitivity for isoprene emission in water-stressed Eucalyptus citriodora saplings. *Journal of Experimental Botany,* **64,** 519-528.

Calfapietra C, Pallozzi E, Lusini I, Velikova V (2013) Modification of BVOC emissions by changes in atmospheric CO_2 and air pollution. In *Biology, controls and models of tree volatile organic compound emissions* (eds Niinemets Ü, Monson RK), pp. In Press. Springer, Berlin.

Calfapietra C, Scarascia Mugnozza G, Karnosky DF, Loreto F, Sharkey TD (2008) Isoprene emission rates under elevated CO_2 and O_3 in two field-grown aspen clones differing in their sensitivity to O_3. *New*

Phytologist, **179,** 55-61.

Calfapietra C, Wiberley AE, Falbel TG*, et al.* (2007) Isoprene synthase expression and protein levels are reduced under elevated O_3 but not under elevated CO_2 (FACE) in field-grown aspen trees. *Plant, Cell and Environment,* **30,** 654-661.

Chow PS, Landhäusser SM (2004) A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology,* **24,** 1129-1136.

Claeys M, Graham B, Vas G*, et al.* (2004) Formation of secondary organic aerosols through photooxidation of isoprene. *Science,* **303,** 1173-1176.

Curtis PS, Wang X (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia,* **113,** 299-313.

de Graaff M-A, van Groenigen K-J, Six J, Hungate B, van Kessel C (2006) Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. *Global Change Biology,* **12,** 2077-2091.

Devi N, Hagedorn F, Moiseev P, Bugmann H, Shiyatov S, Mazepa V, Rigling A (2008) Expanding forests and changing growth forms of Siberian larch at the Polar Urals treeline during the 20th century. *Global Change Biology,* **14,** 1581-1591.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry,* **28,** 350-356.

Eicks M, Maurino V, Knappe S, Flügge U-I, Fischer K (2002) The plastidic pentose phosphate translocator represents a link between the cytosolic and the plastidic pentosephosphate pathways in plants. *Plant Physiology,* **128,** 512-522.

Essemine J, Govindachary S, Ammar S, Bouzid S, Carpentier R (2011) Functional aspects of the photosynthetic light reactions in heat stressed Arabidopsis deficient in digalactosyl-diacylglycerol. *Journal of Plant Physiology,* **168,** 1526-1533.

Fettke J, Malinova I, Albrecht T, Hejazi M, Steup M (2011) Glucose-1-phosphate transport into protoplasts and chloroplasts from leaves of *Arabidopsis*. *Plant Physiology,* **155,** 1723-1734.

Fineschi S, Loreto F, Staudt M, Peñuelas J (2013) Diversification of volatile isoprenoid emissions from trees: evolutionary and ecological perspectives In *Biology, controls and models of tree volatile organic compound emissions* (eds Niinemets Ü, Monson RK), pp. In Press. Springer, Berlin.

Fristedt R, Willig A, Granath P, Crevecoeur M, Rochaix J-D, Vener AV (2009) Phosphorylation of photosystem II controls functional macroscopic folding of photosynthetic membranes in *Arabidopsis*. *Plant Cell,* **21,** 3950-3964.

Funk JL, Mak JE, Lerdau MT (2004) Stress-induced changes in carbon sources for isoprene production in Populus deltoides. *Plant, Cell & Environment,* **27,** 747-755.

Grote R, Monson RK, Niinemets Ü (2013) Leaf-level models of constitutive and stress-driven volatile organic compound emissions. In *Biology, controls and models of tree volatile organic compound emissions* (eds Niinemets Ü, Monson RK), pp. In Press. Springer, Berlin.

Hüve K, Bichele I, Rasulov B, Niinemets Ü (2011) When it is too hot for photosynthesis: heat-induced instability of photosynthesis in relation to respiratory burst, cell permeability changes and H_2O_2 formation. *Plant, Cell and Environment,* **34,** 113-126.

Hüve K, Bichele I, Tobias M, Niinemets Ü (2006) Heat sensitivity of photosynthetic electron transport varies during the day due to changes in sugars and osmotic potential. *Plant, Cell and Environment,* **29,** 212-228.

Hald S, Pribil M, Leister D, Gallois P, Johnson GN (2008) Competition between linear and cyclic electron flow in plants deficient in Photosystem I. *Biochimica Et Biophysica Acta-Bioenergetics,* **1777,** 1173-1183.

Haldimann P, Feller U (2004) Inhibition of photosynthesis by high temperature in oak (Quercus pubescens L.) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant Cell and Environment,* **27,**

1169-1183.

Harley P, Guenther A, Zimmerman P (1996) Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (Liquidambar styraciflua) leaves. *Tree Physiology,* **16,** 25-32.

Heald CL, Wilkinson MJ, Monson RK, Alo CA, Wang G, Guenther A (2009) Response of isoprene emission to ambient CO_2 changes and implications for global budgets. *Global Change Biology,* **15,** 1127-1140.

Jeannette E, Reyss A, Grégory N, Gantet P, Prioul JL (2000) Carbohydrate metabolism in a heat-girdled maize source leaf. *Plant, Cell and Environment,* **23,** 61-69.

Johnson DW (2006) Progressive N limitation in forests: review and implications for long-term responses to elevated CO_2. *Ecology,* **87,** 64-75.

Kant S, Seneweera S, Rodin J, Materne M, Burch D, Rothstein SJ, Spangenberg G (2012) Improving yield potential in crops under elevated CO_2: Integrating the photosynthetic and nitrogen utilization efficiencies. *Frontiers in Plant Science,* **3**.

Kocheva KV, Georgiev GI, Kochev VK (2005) A diffusion approach to the electrolyte leakage from plant tissues. *Physiologia Plantarum,* **125,** 1-9.

Larkindale J, Hall JD, Knight MR, Vierling E (2005) Heat stress phenotypes of arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. *Plant Physiology,* **138,** 882-897.

Laule O, Fürholz A, Chang HS, *et al.* (2003) Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana. Proceedings of the National Academy of Sciences of the United States of America,* **100,** 6866-6871.

Law RD, Crafts-Brandner SJ (1999) Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant Physiology,* **120,** 173-181.

Lehning A, Zimmer I, Steinbrecher R, Bruggemann N, Schnitzler JP (1999) Isoprene synthase activity and its relation to isoprene emission in Quercus robur L-leaves. *Plant Cell and Environment,* **22,** 495-504.

Li D, Chen Y, Shi Y, He X, Chen X (2009) Impact of elevated CO_2 and O_3 concentrations on biogenic volatile organic compounds emissions from *Ginkgo biloba*. *Bulletin of Environmental Contamination and Toxicology,* **82,** 473-477.

Li Z, Ratliff EA, Sharkey TD (2011) Effect of temperature on postillumination isoprene emission in oak and poplar. *Plant Physiology,* **155,** 1037-1046.

Li Z, Sharkey TD (2012) Metabolic profiling of the methylerythritol phosphate pathway reveals the source of post-illumination isoprene burst from leaves. *Plant, Cell and Environment,* **xx,** In Press.

Li Z, Sharkey TD (2013) Molecular and pathway controls on biogenic volatile organic compound emissions. In *Biology, controls and models of tree volatile organic compound emissions* (eds Niinemets Ü, Monson RK), pp. In Press. Springer, Berlin.

Lichtenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annual Review of Plant Physiology and Plant Molecular Biology,* **50,** 47-65.

Lichtenthaler HK (2007) Biosynthesis, accumulation and emission of carotenoids, alpha-tocopherol, plastoquinone, and isoprene in leaves under high photosynthetic irradiance. *Photosynthesis Research,* **92,** 163-179.

Livingston D. I, Hincha D, Heyer A (2009) Fructan and its relationship to abiotic stress tolerance in plants. *Cellular and Molecular Life Sciences,* **66,** 2007-2023.

Loreto F, Schnitzler J-P (2010) Abiotic stresses and induced BVOCs. *Trends in plant science,* **15,** 154-166.

Loreto F, Velikova V (2001) Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology,* **127,** 1781-1787.

Luo H-B, Ma L, Xi H-F, *et al.* (2011) Photosynthetic responses to heat treatments at different temperatures and following recovery in grapevine (Vitis amurensis L.) leaves. *PLoS ONE,* **6,** e23033-e23033.

Luo Y (2007) Terrestrial carbon-cycle feedback to climate warming. In *Annual Review of Ecology Evolution and Systematics* Vol. 38, pp. 683-712.

Luo Y, Sims DA, Griffin KL (1997) Nonlinearity of photosynthetic responses to growth in rising atmospheric CO_2: an experimental and modelling study. *Global Change Biology,* **3,** 173-183.

Makino A, Mae T (1999) Photosynthesis and plant growth at elevated levels of CO2. *Plant and Cell Physiology,* **40,** 999-1006.

Miyazawa S-I, Warren CR, Turpin DH, Livingston NJ (2011) Determination of the site of CO_2 sensing in poplar: is the area-based N content and anatomy of new leaves determined by their immediate CO_2 environment or by the CO_2 environment of mature leaves? *Journal of Experimental Botany,* **62,** 2787-2796.

Monson RK (2013) Metabolic and gene expression controls on the production of biogenic volatile organic compounds. In *Biology, controls and models of tree volatile organic compound emissions* (eds Niinemets Ü, Monson RK), pp. In Press. Springer, Berlin.

Monson RK, Grote R, Niinemets Ü, Schnitzler J-P (2012a) Tansley review. Modeling the isoprene emission rate from leaves. *The New Phytologist,* **195,** 541-559.

Monson RK, Jones RT, Rosenstiel TN, Schnitzler J-P (2013) Why only some plants emit isoprene. *Plant, Cell and Environment,* **36,** 503-516..

Myers DA, Thomas RB, DeLucia EH (1999) Photosynthetic responses of loblolly pine (*Pinus taeda*) needles to experimental reduction in sink demand. *Tree Physiology,* **19,** 235-242.

Nagao M, Minami A, Arakawa K, Fujikawa S, Takezawa D (2005) Rapid degradation of starch in chloroplasts and concomitant accumulation of soluble sugars associated with ABA-induced freezing tolerance in the moss *Physcomitrella patens*. *Journal of Plant Physiology,* **162,** 169-180.

Niinemets Ü (1999) Research review. Components of leaf dry mass per area - thickness and density - alter leaf photosynthetic capacity in reverse directions in woody plants. *The New Phytologist,* **144,** 35-47.

Niinemets Ü (2012) Whole plant photosynthesis. In *Terrestrial photosynthesis in a changing environment. A molecular, physiological and ecological approach* (eds Flexas J, Loreto F, Medrano H), pp. 399-423. Cambridge University Press, Cambridge.

Niinemets Ü, Monson RK, Arneth A, *et al.* (2010) The leaf-level emission factor of volatile isoprenoids: caveats, model algorithms, response shapes and scaling. *Biogeosciences,* **7,** 1809-1832.

Niinemets Ü, Oja V, Kull O (1999a) Shape of leaf photosynthetic electron transport versus temperature response curve is not constant along canopy light gradients in temperate deciduous trees. *Plant, Cell and Environment,* **22,** 1497-1514.

Niinemets Ü, Reichstein M (2003) Controls on the emission of plant volatiles through stomata: sensitivity or insensitivity of the emission rates to stomatal closure explained. *Journal of Geophysical Research - Atmospheres,* **108,** 4208, doi:4210.1029/2002JD002620.

Niinemets Ü, Tenhunen JD, Harley PC, Steinbrecher R (1999b) A model of isoprene emission based on energetic requirements for isoprene synthesis and leaf photosynthetic properties for *Liquidambar* and *Quercus*. *Plant, Cell and Environment,* **22,** 1319-1336.

Nishiyama Y, Allakhverdiev SI, Murata N (2006) A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II. *Biochimica Et Biophysica Acta-Bioenergetics,* **1757,** 742-749.

Nowak RS, Ellsworth DS, Smith SD (2004) Functional responses of plants to elevated atmospheric CO_2 - do photosynthetic and productivity data from FACE experiments support early predictions? *The New Phytologist,* **162,** 253-280.

Owen SM, Peñuelas J (2005) Opportunistic emissions of volatile isoprenoids. *Trends in plant science,* **10,**

420-426.

Peñuelas J, Llusià J, Asensio D, Munné-Bosch S (2005) Linking isoprene with plant thermotolerance, antioxidants and monoterpene emissions. *Plant, Cell and Environment,* **28,** 278-286.

Pfeiffer S, Krupinska K (2005) New Insights in Thylakoid Membrane Organization. *Plant and Cell Physiology,* **46,** 1443-1451.

Possell M, Hewitt CN (2011) Isoprene emissions from plants are mediated by atmospheric CO_2 concentrations. *Global Change Biology,* **17,** 1595-1610.

Qian H, Joseph R, Zeng N (2010) Enhanced terrestrial carbon uptake in the Northern High Latitudes in the 21st century from the Coupled Carbon Cycle Climate Model Intercomparison Project model projections. *Global Change Biology,* **16,** 641-656.

Rasulov B, Copolovici L, Laisk A, Niinemets Ü (2009a) Postillumination isoprene emission: in vivo measurements of dimethylallyldiphosphate pool size and isoprene synthase kinetics in aspen leaves. *Plant Physiology,* **149,** 1609-1618.

Rasulov B, Hüve K, Bichele I, Laisk A, Niinemets Ü (2010) Temperature response of isoprene emission in vivo reflects a combined effect of substrate limitations and isoprene synthase activity: a kinetic analysis. *Plant Physiology,* **154,** 1558-1570.

Rasulov B, Hüve K, Laisk A, Niinemets Ü (2011) Induction of a longer-term component of isoprene release in darkened aspen leaves: origin and regulation under different environmental conditions. *Plant Physiology,* **156,** 816-831.

Rasulov B, Hüve K, Välbe M, Laisk A, Niinemets Ü (2009b) Evidence that light, carbon dioxide and oxygen dependencies of leaf isoprene emission are driven by energy status in hybrid aspen. *Plant Physiology,* **151,** 448-460.

Rosenkranz M, Schnitzler J-P (2013) Genetic engineering of BVOC emissions from trees. In *Biology, controls and models of tree volatile organic compound emissions* (eds Niinemets Ü, Monson RK), pp. In Press. Springer, Berlin.

Sanadze (2004) Biogenic Isoprene (A Review). *Russian Journal of Plant Physiology,* **2004, Volume 51,** 13.

Sanadze G (2010) Photobiosynthesis of isoprene as an example of leaf excretory function in the light of contemporary thermodynamics. *Russ J Plant Physiol,* **57,** 1-6.

Sanz-Saez A, Erice G, Aranjuelo I, Nogues S, Irigoyen JJ, Sanchez-Diaz M (2010) Photosynthetic down-regulation under elevated CO_2 exposure can be prevented by nitrogen supply in nodulated alfalfa. *Journal of Plant Physiology,* **167,** 1558-1565.

Saxe H, Ellsworth DS, Heath J (1998) Tansley review No. 98. Tree and forest functioning in an enriched CO_2 atmosphere. *The New Phytologist,* **139,** 395-436.

Schwender J, Zeidler J, Gröner R, *et al.* (1997) Incorporation of 1-deoxy-D-xylulose into isoprene and phytol by higher plants and algae. *FEBS Letters,* **414,** 129-134.

Scotti Campos P, Quartin V, Cochicho Ramalho J, Nunes MA (2003) Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of *Coffea* sp. *Journal of Plant Physiology,* **160,** 283-292.

Sharkey TD (2005) Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant Cell and Environment,* **28,** 269-277.

Sharkey TD, Chen XY, Yeh S (2001a) Isoprene increases thermotolerance of fosmidomycin-fed leaves. *Plant Physiology,* **125,** 2001-2006.

Sharkey TD, Gray DW, Pell HK, Breneman SR, Topper L (2012) Isoprene synthase genes form a monophyletic clade of acyclic terpene synthases in the Tps-b terpene synthase family. *Evolution,* **xx,** xx-xx, doi:10.1111/evo.12013.

Sharkey TD, Loreto F, Delwiche CF (1991) High carbon dioxide and sun/shade effects on isoprene emission

from oak and aspen tree leaves. *Plant, Cell and Environment,* **14,** 333-338.

Sharkey TD, Yeh S (2001b) Isoprene emission frm plants. *Annual Review of Plant Physiology and Plant Molecular Biology,* **52,** 407-436.

Sims DA, Seemann JR, Luo Y (1998a) Elevated CO_2 concentration has independent effects on expansion rates and thickness of soybean leaves across light and nitrogen gradients. *Journal of Experimental Botany,* **49,** 583-591.

Sims DA, Seemann JR, Luo Y (1998b) The significance of differences in the mechanisms of photosynthetic acclimation to light, nitrogen and CO_2 for return on investment in leaves. *Functional Ecology,* **12,** 185-194.

Singsaas EL, Lerdau M, Winter K, Sharkey TD (1997) Isoprene increases thermotolerance of isoprene-emitting species. *Plant Physiology,* **115,** 1413-1420.

Singsaas EL, Sharkey TD (2000) The effects of high temperature on isoprene synthesis in oak leaves. *Plant, Cell & Environment,* **23,** 751-757.

Siwko ME, Marrink SJ, de Vries AH, Kozubek A, Uiterkamp A, Mark AE (2007) Does isoprene protect plant membranes from thermal shock? A molecular dynamics study. *Biochimica Et Biophysica Acta-Biomembranes,* **1768,** 198-206.

Sun Z, Copolovici L, Niinemets Ü (2012a) Can the capacity for isoprene emissions acclimate to environmental modifications during autumn senescence in temperate deciduous tree species *Populus tremula*? *Journal of Plant Research,* **125,** 263-274.

Sun Z, Niinemets Ü, Hüve K, Noe SM, Rasulov B, Copolovici L, Vislap V (2012b) Enhanced isoprene emission capacity and altered light responsiveness in aspen grown under elevated atmospheric CO_2 concentration. *Global Change Biology,* **18,** 3423-3440.

Sun Z, Niinemets Ü, Hüve K, Rasulov B, Noe SM (2013) Elevated atmospheric CO_2 concentration leads to increased whole-plant isoprene emission in hybrid aspen (*Populus tremula* x *P. tremuloides*). *The New Phytologist,* **198,** 788-800.

Suzuki H, Taguchi Y, Sugiura M, Boussac A, Noguchi T (2006) Structural perturbation of the carboxylate ligands to the manganese cluster upon Ca2+/Sr2+ exchange in the S-state cycle of photosynthetic oxygen evolution as studied by flash-induced FTIR difference spectroscopy. *Biochemistry,* **45,** 13454-13464.

Taub DR, Seemann JR, Coleman JS (2000) Growth in elevated CO_2 protects photosynthesis against high-temperature damage. *Plant Cell and Environment,* **23,** 649-656.

cherkez GGB, Farquhar GD, Andrews TJ (2006) Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. *Proceedings of the National Academy of Sciences of the United States of America,* **103,** 7246-7251.

Toth SZ, Nagy V, Puthur JT, Kovacs L, Garab G (2011) The physiological role of ascorbate as photosystem II electron donor: protection against photoinactivation in heat-stressed leaves. *Plant Physiology,* **156,** 382-392.

Tripp KE, Peet MM, Pharr DM, Willits DH, Nelson PV (1991) CO_2-enhanced yield and foliar deformation among tomato genotypes in elevated CO_2 environments. *Plant Physiology,* **96,** 713-719.

Vahala J, Keinänen M, Schützendübel A, Polle A, Kangasjärvi J (2003) Differential effects of elevated ozone on two hybrid aspen genotypes predisposed to chronic ozone fumigation. Role of ethylene and salicylic acid. *Plant Physiology,* **132,** 196-205.

Velikova V, Loreto F (2005) On the relationship between isoprene emission and thermotolerance in *Phragmites australis* leaves exposed to high temperatures and during the recovery from a heat stress. *Plant, Cell & Environment,* **28,** 318-327.

Velikova V, Loreto F, Tsonev T, Brilli F, Edreva A (2006) Isoprene prevents the negative consequences of high temperature stress in Platanus orientalis leaves. *Functional Plant Biology,* **33,** 931-940.

Velikova V, Varkonyi Z, Szabo M*, et al.* (2011) Increased thermostability of thylakoid membranes in isoprene-emitting leaves probed with three biophysical techniques. *Plant Physiology,* **157,** 905-916.

Vickers CE, Gershenzon J, Lerdau MT, Loreto F (2009) A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nature Chemical Biology,* **5,** 283-291.

Vickers CE, Possell M, Hewitt CN, Mullineaux PM (2010) Genetic structure and regulation of isoprene synthase in Poplar (Populus spp.). *Plant Molecular Biology,* **73,** 547-558.

Wilkinson MJ, Monson RK, Trahan N*, et al.* (2009) Leaf isoprene emission rate as a function of atmospheric CO_2 concentration. *Global Change Biology,* **15,** 1189-1200.

Yin L, Lundin B, Bertrand M*, et al.* (2010) Role of thylakoid ATP/ADP carrier in photoinhibition and photoprotection of photosystem II in arabidopsis. *Plant Physiology,* **153,** 666-677.

Yoshioka M, Uchida S, Mori H*, et al.* (2006) Quality control of photosystem II - Cleavage of reaction center D1 protein in spinach thylakoids by FtsH protease under moderate heat stress. *Journal of Biological Chemistry,* **281,** 21660-21669.

Zhu S-Q, Yu C-M, Liu X-Y, Ji B-H, Jiao D-M (2007) Changes in unsaturated levels of fatty acids in thylakoid PSII membrane lipids during chilling-induced resistance in rice. *Journal of Integrative Plant Biology,* **49,** 463-471.

Figure captions

Figure 1. Transmission electron microscopy (TEM) images of leaf palisade mesophyll cells in hybrid aspen (*Populus tremula* x *P. tremuloides*) leaves developed under the ambient CO_2 concentration of 380 μmol mol^{-1} (a) and elevated CO_2 concentration of 780 μmol mol^{-1} (b). The cells were viewed at 2100 x magnification with a Philips Tecnai 10 TEM microscope (FEI, Eindhoven, Netherlands) using an accelerating voltage of 80 kV.

Figure 2. Temperature response of net assimilation rate (a, b), and isoprene emission rate (c, d) in hybrid aspen leaves under different growth and measurement CO_2 environments and at different light intensities. Data in (a) and (c) correspond to measurements under a moderate light intensity of 500 μmol m^{-2} s^{-1}, and (b) and (d) to measurements under a strong light intensity of 2000 μmol m^{-2} s^{-1}. Ambient (380) and Elevated (380) denote plants grown under the ambient [CO_2] of 380 μmol mol^{-1} and elevated [CO_2] of 780 μmol mol^{-1}, and both measured at [CO_2] of 380 μmol mol^{-1}. Ambient (780) and Elevated (780) label plants grown under the ambient [CO_2] of 380 μmol mol^{-1} and elevated [CO_2] of 780 μmol mol^{-1}, and both measured at [CO_2] of 780 μmol mol^{-1}. Data are averages (+ SE) of 8-10 replicate leaves. At each individual temperature, different letters on the top of each bar indicate statistically significant differences at given temperature ($P < 0.05$).

Figure 3. Temperature response of isoprene concentration in leaf intercellular air space ($C_{iso,i}$) (a, b) (Eq. 1) and the percentage of carbon lost as isoprene (c, d) in hybrid aspen leaves grown under different growth CO_2 environments and measured under different growth and light conditions. Data are averages (+ SE) of 8-10 replicate leaves. Data presentation and statistics as in Fig. 2.

Figure 4. Relative reduction of net assimilation rate (Eq. 2) (a, b) and relative increase of isoprene emission rate (Eq. 3) (c, d) with increasing temperature in hybrid aspen leaves grown under different [CO_2] of 380 μmol mol^{-1} (Ambient) and 780 μmol mol^{-1} (Elevated) and measured under different [CO_2] and light conditions. Measurement CO_2 concentration, 380 μmol mol^{-1} or 780 μmol mol^{-1} is shown in parentheses. Data are averages (± SE) of 8-10

replicate leaves.

Figure 5. Relationships of the decrease of net assimilation rate (Eq. 2) with the reduction in the effective quantum yield of PSII over the temperature range of 30-50 ^{o}C (n = 8-10 for individual data points). Temperature responses of the change in net assimilation rate are demonstrated in Fig. 4a, c. The measurements conducted at light intensities of 500 μmol m^{-2} s^{-1} and 2000 μmol m^{-2} s^{-1} were pooled. The decrease of PSII quantum yield was calculated as $(Y_{30}-Y_T)/Y_{30}$, where Y_{30} is the yield at 30 ^{o}C and Y_T the PSII yield at any other measurement temperature between 30-50 ^{o}C. Error bars denote ± SE. Data were fitted by linear regressions.

Figure 6. Correlations of the decrease of net assimilation rate (R_A, Eq. 2) with increase of isoprene emission rate (R_I) during the heat stress in hybrid aspen leaves under different growth (ambient of 380 μmol mol^{-1} and elevated of 780 μmol mol^{-1}) and measurement CO_2 conditions (380 vs. 780 μmol mol^{-1}). The measurements were conducted under a moderate light intensity of 500 μmol m^{-2} s^{-1} (a) and under a strong light intensity of 2000 μmol m^{-2} s^{-1} (b). Average (± SE) values for 8-10 replicate leaves are demonstrated. Linear trendlines are shown to highlight the trends. Differences among the relationships were analyzed using covariation analyses with all replicate measurements included (see the Results).

Figure 7. Average (+SE) relative leaf electrolyte leakage in response to heat stress (a), and correlations of the decrease of net assimilation rate (see Fig. 4a, c) with leaf electrical conductivity at 50 ^{o}C (b) in hybrid aspen leaves grown under ambient [CO_2] of 380 μmol mol^{-1} and elevated [CO_2] of 780 μmol mol^{-1}. In (a), the data are averages of 8-10 replicate leaves and averages with different letters are significantly different at $P < 0.05$ according to one-way ANOVA. In (b), the data correspond to individual measurements, and the measurements conducted at light intensities of 500 μmol m^{-2} s^{-1} and 2000 μmol m^{-2} s^{-1} were pooled. Data in (b) were fitted by linear regressions.

Table 1. Foliage anatomical, morphological and chemical traits of hybrid aspen (*Populus tremula* x *P. tremuloides*) trees grown under ambient (380 μmol mol^{-1}) and elevated (780 μmol mol^{-1}) atmospheric CO_2 concentrations

Trait	Treatment		*P*
	Ambient	Elevated	
Leaf fresh mass per unit leaf area (g m^{-2}) (M_F)	153.5 ± 3.7	180.9±4.7	<0.0001
Mass of water per leaf area (g m^{-2}) (M_{WA})	125 ± 5	142.4 ±3.2	0.001
Mass of water per leaf volume (g cm^{-3}) (M_{WV})	0.713 ± 0.040	0.728 ± 0.017	0.76
Percentage of intercellular air space (%) (f_{ias})	26.2 ± 1.1	24.3 ± 1.1	0.21
Percentage of mesophyll cells of total leaf volume (without air spaces) (%) ($f_{t,mes}$, Eq. 6)	59.2 ± 2.1	61.4 ± 3.4	0.25
Percentage of chloroplasts (without air spaces) (%) (f_{Chl})	11.4 ±1.3	29 ± 5	0.02
Percentage of leaf water in mesophyll (%) ($F_{W,mes}$, Eq. 7)	46.1 ± 3.0	51.0 ± 2.4	0.25
Percentage of leaf water in chloroplasts (%) ($F_{W,Chl}$, Eq. 8)	7.72 ± 0.45	13.0 ± 1.1	0.004
Sugar content in leaf water (g g^{-1}) (S_W)	0.036 ± 0.007	0.053 ±0.008	0.002
Sugar content per dry mass (g g^{-1}) (S_D)	0.1293 ± 0.0035	0.1567 ± 0.0033	<0.0001

Data are means ± SE of four independent samples (trees). Means were compared using ANOVA. Leaf dry mass per unit area was 28.8 ±0.6 g m^{-2} for plants grown under ambient and 38.6 ± 0.8 g m^{-2} for plants grown under elevated [CO_2] ($P < 0.001$) (Sun *et al.*, 2012b).

Fig. 1

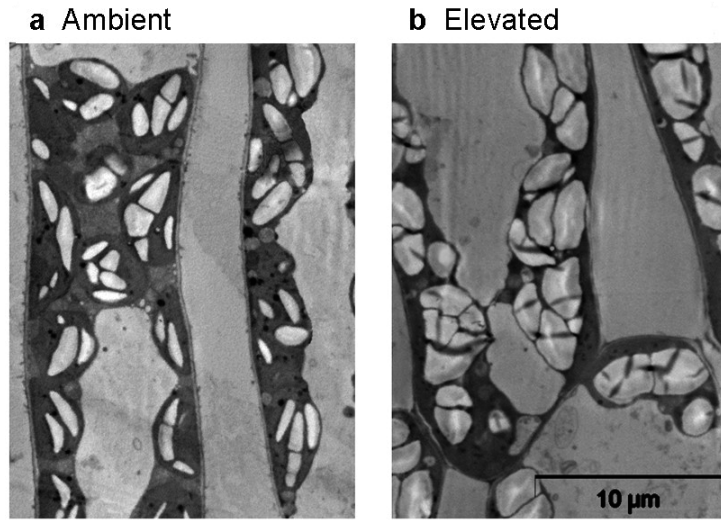

a Ambient
b Elevated
10 µm

Fig.2

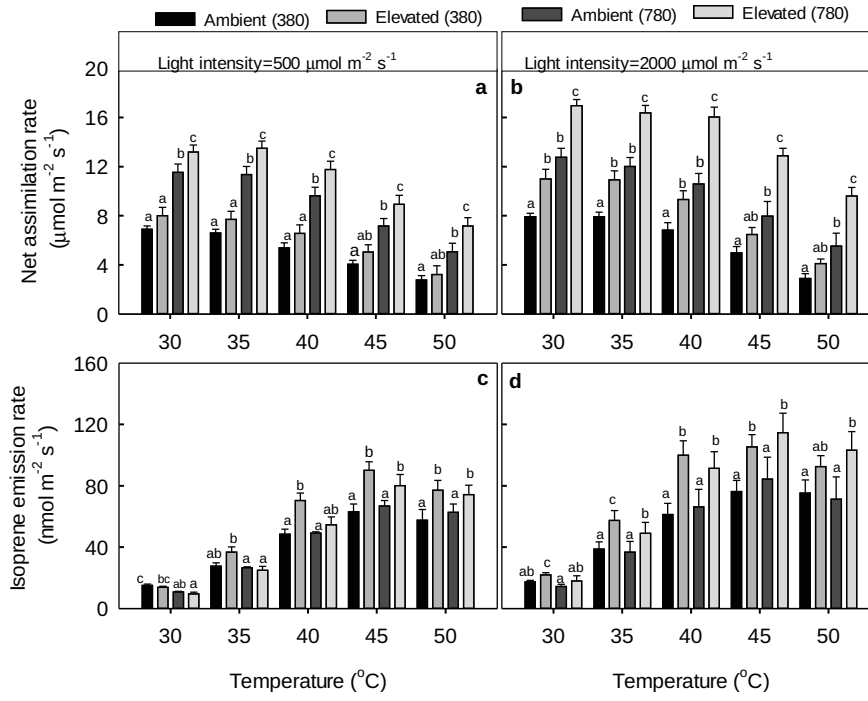

Ambient (380)
Elevated (380)
Ambient (780)
Elevated (780)
Light intensity=500 µmol m-2 s-1
Light intensity=2000 µmol m-2 s-1
Net assimilation rate (µmol m-2 s-1)
Isoprene emission rate (nmol m-2 s-1)
Temperature (°C)
Temperature (°C)
a
b
c
d

Fig. 3

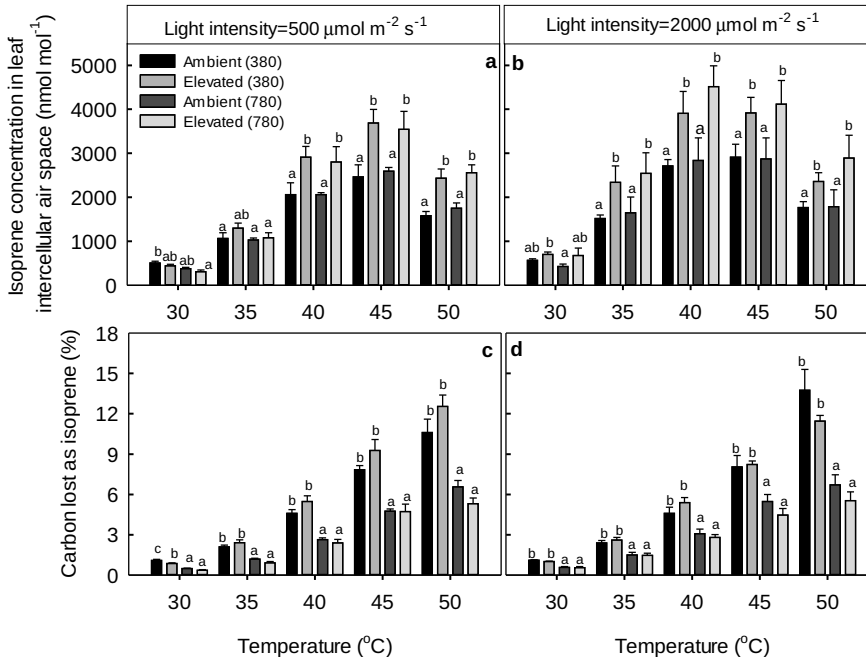

Light intensity=500 μmol m-2 s-1
Light intensity=2000 μmol m-2 s-1
Ambient (380)
Elevated (380)
Ambient (780)
Elevated (780)
Isoprene concentration in leaf intercellular air space (nmol mol-1)
Carbon lost as isoprene (%)
Temperature (°C)
Temperature (°C)

Fig. 4

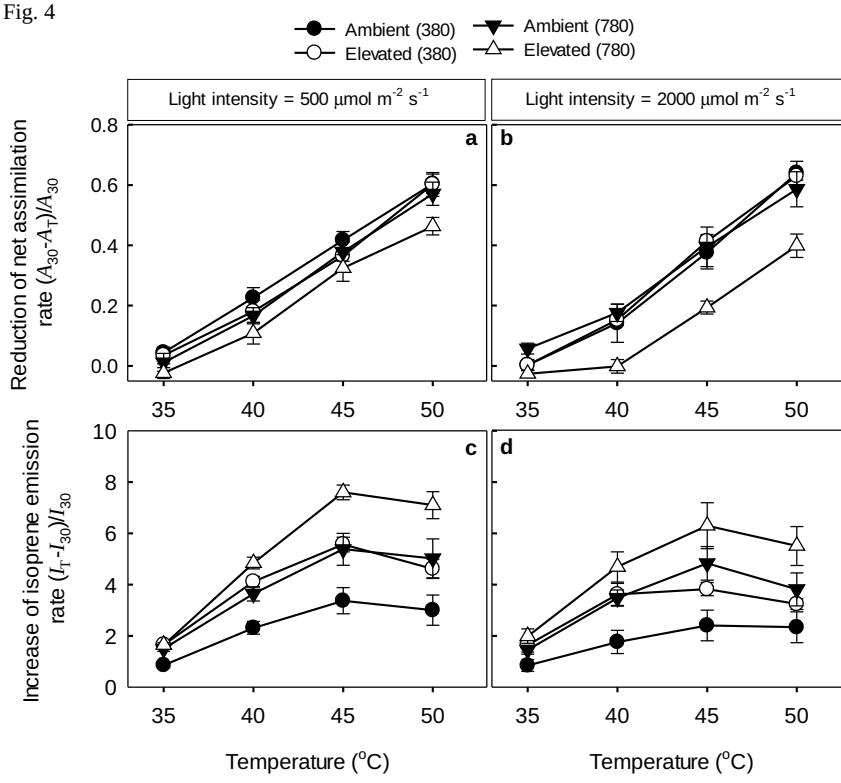

Ambient (380)
Elevated (380)
Ambient (780)
Elevated (780)
Light intensity = 500 μmol m-2 s-1
Light intensity = 2000 μmol m-2 s-1
Reduction of net assimilation rate (A30-AT)/A30
Increase of isoprene emission rate (IT-I30)/I30
Temperature (°C)
Temperature (°C)

Fig. 5

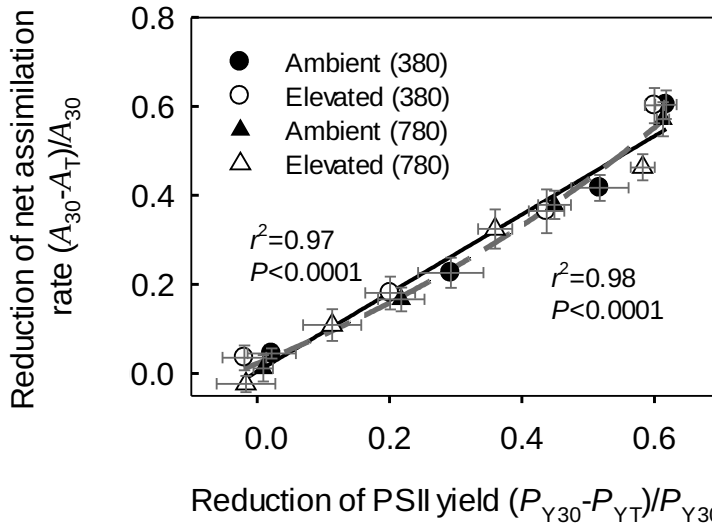


Fig. 6

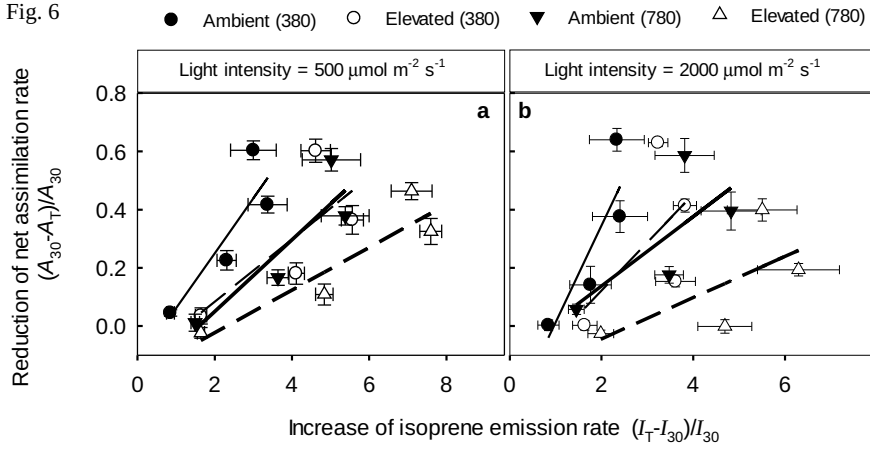
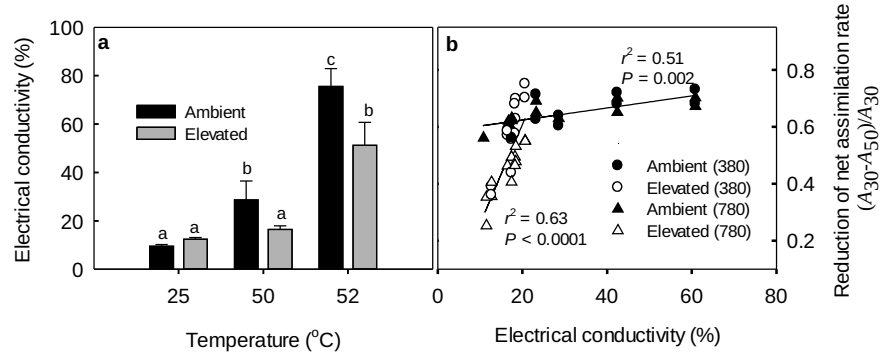


Fig. 7



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REGULAR PAPER

Can the capacity for isoprene emission acclimate to environmental modifications during autumn senescence in temperate deciduous tree species *Populus tremula*?

Zhihong Sun · Lucian Copolovici · Ülo Niinemets

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Abstract Changes in isoprene emission ($\Phi_{isoprene}$), and foliage photosynthetic (A) rates, isoprene precursor dimethylallyldiphosphate (DMADP), and nitrogen and carbon contents were studied from late summer to intensive leaf fall in *Populus tremula* to gain insight into the emission controls by temperature and endogenous, senescence-induced, modifications. Methanol emissions, characterizing degradation of cell wall pectins, were also measured. A rapid reduction in $\Phi_{isoprene}$ and A of 60–70% of the initial value was observed in response to a rapid reduction of ambient temperature by ca. 15°C (cold stress). Later phases of senescence were associated with further reductions in $\Phi_{isoprene}$ and A, with simultaneous major decrease in nitrogen content. However, during episodes of temperature increase, A and in particular, $\Phi_{isoprene}$ partly recovered. Variation in $\Phi_{isoprene}$ during senescence was correlated with average temperature of preceding days, with the highest degree of explained variance observed with average temperature of 6 days. Throughout the study, methanol emissions were small, but a large burst of methanol emission was associated with leaf yellowing and abscission. Overall, these data demonstrate that the capacity for isoprene emission can adjust to environmental conditions in senescing leaves as well, but the responsiveness is low compared with mid-season and is also affected by stress.

Keywords Emission modelling · Isoprene emission · Methanol emission · Nitrogen · Photosynthesis · Seasonality · Temperature acclimation

Z. Sun · L. Copolovici · Ü. Niinemets (✉)
Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, 51014 Tartu, Estonia
e-mail: ylo@emu.ee

Introduction

Isoprene is a key emitted volatile plant isoprenoid that is assumed to serve as an important lipid-soluble antioxidant in leaves (Vickers et al. 2009), protecting against reactive oxygen species (ROS) formed in response to a variety stresses such as heat or ozone stress (Affek and Yakir 2002; Loreto and Velikova 2001; Sharkey et al. 2008; Velikova and Loreto 2005). Isoprene emission is regulated both by immediate variations in key environmental drivers light and temperature and by longer-term seasonal modifications in isoprene emission capacity (E_S), i.e., the emission rate standardized for immediate variations in light and temperature (commonly measured at 1,000 μmol m^{-2} s^{-1} incident light and at a leaf temperature of 30°C) (for reviews see, Niinemets et al. 2010a, c). The rapid responses of isoprene emission to light and temperature are thought to result from changes in the supply of intermediates for isoprene synthesis (Loreto and Sharkey 1993; Magel et al. 2006; Rasulov et al. 2010, 2011, 2009b; Schnitzler et al. 2004). The longer term changes, increases in the emission in young leaves and decreases in old leaves, are thought to reflect modifications in the expression of isoprene synthase and the enzymes of the chloroplastic MEP (2-C-methyl-D-erythritol 4-phosphate) pathway that is responsible for isoprene synthesis (Mayrhofer et al. 2005; Sharkey and Yeh 2001; Wiberley et al. 2008, 2009, 2005). So far, ontogenetic modifications in E_S have been simulated using empirical models where E_S depends on leaf age (Arneth et al. 2008; Grote et al. 2010; Schnitzler et al. 1997).

Apart from ontogenetic modifications, E_S acclimates to day-to-day variations in temperature environment, and changes in isoprene emission in non-senescent leaves can be predicted on the basis of past temperature environment, typically on the basis of the temperature of a few preceding

days (Geron et al. 2000; Guenther et al. 2006; Pétron et al. 2001; Sharkey et al. 1999; Wiberley et al. 2008). As temperature also varies during the season, attempts have been made to use the relationship of E_S on past temperature to simulate seasonality in isoprene emission (Guenther et al. 2006; Lehning et al. 2001). Typically, a constant response function of E_S versus past average temperature is used for the entire season (Guenther et al. 2006; Lehning et al. 2001). Use of such a simplification rests on the assumption that the responsiveness of E_S to past temperature is invariable throughout the season. However, the validity of this assumption during autumn senescence has not been tested.

Autumn senescence in temperate trees is a coordinated break-down of cellular structures, and degradation of key proteins, resulting in gradual reduction of foliage physiological activities, in particular in decreases in photosynthesis, and resorption of limiting mineral elements such as N and P for the use in the next growing season (Andersson et al. 2004; Keskitalo et al. 2005; Niinemets et al. 2004). However, leaf senescence in temperate trees is a time-consuming event, taking 30–70 days from the first signs of senescence until leaf abscission (Fracheboud et al. 2009; Grassi et al. 2005; Herrick and Thomas 2003; Niinemets et al. 2004; Niinemets and Tamm 2005; Reich et al. 1991). During this period, environmental conditions fluctuate between days and weeks, and the question is to which extent foliage isoprene emission capacity can track the changes in ambient temperature fluctuations at the background of overall dismantling of proteins, resorption of N and reduction of photosynthetic potentials. Furthermore, cold stress events that are frequent in autumn can lead to rapid reductions in foliage photosynthetic function (DeLucia 1987; DeLucia and Smith 1987; Falge et al. 1996; Rosenthal and Camm 1997), but the way E_S dependence on past temperature is modified by such stress events during senescence has not been studied. Depending on the responsiveness of isoprene emission capacity to ambient conditions, spells of warm weather during senescence may either have strong or only marginal effects on atmospheric chemistry.

Senescence also involves activation of several genes, but these are commonly genes associated with proteolysis and degradation of cellular structures (Andersson et al. 2004; Hopkins et al. 2007; Kontunen-Soppela et al. 2010). On the other hand, activation of catabolic processes can result in formation of highly reactive compounds, e.g., highly reactive intermediates of chlorophyll catabolism (Matile et al. 1992). Given further the overall reduction in photosynthetic activity, enhancing potentially the sensitivity of foliage to photoinhibition (Adams et al. 1990; Hoch et al. 2001; Merzlyak and Gitelson 1995), enhanced accumulation of reactive oxygen species (ROS) typically occurs in senescing leaves (Procházková and Wilhelmová 2007; Yoshida 2003; Zapata et al. 2005; Zentgraf and Hemleben 2008), potentially jeopardizing coordinated dismantling of cellular structures and retranslocation of mineral nutrients. To cope with potentially higher oxidative stress, synthesis of several antioxidants such as flavonoids increases in senescing leaves (Feucht et al. 1997; Garcia-Plazaola et al. 2003; Hoch et al. 2001; Keskitalo et al. 2005). In addition, flavonoid pigments absorbing in visible spectral region of solar radiation, anthocyanins, in particular, also can serve as light screens reducing photooxidative damage (Garcia-Plazaola et al. 2003; Hoch et al. 2001). From the perspective of enhanced oxidative stress during senescence, isoprene, capable of detoxification of ROS can further amplify leaf antioxidative capacity. If so, changes in E_S during leaf senescence may not be entirely due to overall monotonous reduction of leaf functional activity, but plants may actively change E_S through leaf senescence in response to environmental variations. While in mid-season, average temperature of preceding days has been proven to be the best predictor of E_S (Geron et al. 2000; Guenther et al. 2006; Pétron et al. 2001; Sharkey et al. 1999; Wiberley et al. 2008), minimum temperatures may constitute a stronger stress factor during senescence. In fact, enhancement of isoprene synthase activity by low night temperatures has been reported (Ibrahim et al. 2010). On the other hand, as all chemical reactions run faster at higher temperature, formation of ROS can depend on temperature per se not necessarily on minimum temperature.

We studied modifications in foliage photosynthetic activity and isoprene emission rate from fully active non-senescent leaves until intensive leaf fall in broad-leaved deciduous isoprene-emitting species *Populus tremula* L. to understand how the emission controls are shared between temperature environment and senescence. The signals of past average, minimum and maximum temperature on E_S were tested. We hypothesized that the control by past temperature on isoprene emission is weakened in senescing leaves, and thus, foliage photosynthesis and isoprene emission rate decrease in a coordinated manner through leaf senescence. We also hypothesized that minimum temperatures exert a stronger control on the emission than average temperatures. Foliage nitrogen content and pool size of immediate isoprene precursor, dimethylallyldiphosphate (DMADP), was also monitored to gain insight into the controls of emission activity by precursor pool size and N availability for protein synthesis. Methanol emissions that are elicited as the result of activation of pectin methylesterases during oxidative stress (Beauchamp et al. 2005; Cojocariu et al. 2006; Micheli 2001) were also studied throughout leaf senescence.

Materials and methods

Study site and foliage sampling

The study was conducted in 2008 in the vicinity of Tartu, Estonia (58.39°N, 26.70°E, elevation 41 m). The site supports naturally-regenerated 8–10 years old and 8–12 m tall *Populus tremula* trees. The samples of foliage for gas-exchange measurements were taken from the non-exposed side of the edge trees of *P. tremula* at a height of 2–3 m receiving about 25% of above-canopy solar radiation. Foliage sampling was carried out at 900 h in every 3–9 days between August 24 (fully active dark green leaves) and October 13 (yellowed leaves close to abscission, intensive leaf fall in the canopy). According to previous measurements, foliage photosynthetic characteristics and isoprene emission rates were stable and close to maximum (90–95%) in late summer (mid August to early September) (Niinemets et al. 2010b; 2004). The selected twigs were cut under water and transported immediately to the laboratory for gas-exchange measurements. At each sampling event, three independent samples from neighbouring trees were taken. Different branches were sampled at different dates to minimize the impact of twig excision on volatile emissions.

CO_2 and water vapour exchange, isoprene and methanol emission measurements

Gas-exchange and trace gas measurements were conducted by a custom-made dual-channel gas exchange system (Copolovici and Niinemets 2010 for a detailed description). The system has a thermostatted 1.2 L glass cuvette, the gas flow rate through the system is maintained at 1.4 L min^{-1}, and the air inside the chamber is vigorously mixed by a fan installed in the chamber. In these experiments, the chamber was thermostatted at 30°C (leaf temperature 30 ± 1°C).

After collection, the twigs were stabilized at 25°C for 2–3 h in dim light. Following the stabilization period, the twig with 3–5 leaves was enclosed in the chamber, light provided by four 65 W Osram Decostar dichroic halogen lamps was switched on and kept at 600 ± 100 μmol m^{-2} s^{-1} incident to leaf surface. Foliage CO_2 and water vapour exchange rates were monitored by an infra-red dual-channel gas analyzer operated in differential mode (CIRAS II, PP-systems, Amesbury, MA, USA), and measurements were taken when stomata opened and gas exchange rates reached a steady-state level, typically 30 min to 1 h after enclosure of the leaves in the chamber.

The air for the measurements was drawn by a pump from outside, passed though a 15 L buffer volume and an ozone trap consisting of a 50 cm copper tube (i.d. 5 mm) activated by concentrated hydrochloric acid. Ozone trapping is based on the catalytic activity of Cu(II) compounds, in particular, $CuCl_2$ formed in the reaction with HCl from CuO deposited on the copper tube walls (Rakitskaya et al. 2006; Spasova et al. 2007). In our setup, ambient ozone concentrations were reduced from the level of 25–50 nmol mol^{-1} to ca. 1 nmol mol^{-1} by the trap. CO_2 concentration in the chamber was 380 ± 20 μmol mol^{-1} in these experiments.

After steady-state CO_2 and H_2O exchange rates were achieved, methanol and isoprene concentrations were measured by a Proton Transfer Reaction-Mass Spectrometer (PTR-MS) (High sensitivity version, Ionicon GmbH, Innsbruck, Austria, for detailed description see Hansel et al. 1995; Lindinger et al. 1998a, b) as in Copolovici and Niinemets (2010). Methanol was detected as protonated parent ion at m/z of 33, and isoprene as m/z of 69. A standard certified gas mixture (lot 3821, Ionimed Analytik, Innsbruck, Austria) was used to calibrate PTR-MS. Incoming and outgoing isoprene and methanol concentrations were measured switching the air flows of the two channel gas exchange system between the reference and sample lines (Copolovici and Niinemets 2010). Although volatile products of the lipoxygenase pathway (LOX products) can also provide important insight into the physiological status of the leaves during senescence and stress (e.g., Beauchamp et al. 2005), twig cutting may importantly enhance their emission (Vuorinen et al. 2005), and therefore, LOX products were not investigated in the current study. In the case of isoprene and methanol, no effect of twig cutting on the emission rates was observed in the steady-state in control experiments with potted *P. tremula* plants when the same protocol was followed for cut and uncut twigs.

Foliage gas-exchange rates were calculated according to von Caemmerer and Farquhar (1981) and trace gas exchange rates according to Copolovici and Niinemets (2010).

Foliage structural and chemical analyses

At the end of each measurement, leaves were scanned with a resolution of 300 dpi, and the area was measured from digital images with UTHSCA Imagetool 2.00alpha (The University of Texas Health Science Center, San Antonio, TX, USA). Leaf fresh mass was determined immediately after leaf scanning and leaf dry mass was estimated after oven-drying for 48 h at 70°C. From these measurements, leaf dry to fresh mass ratio (D_F) and leaf dry mass per unit area (M_A) were calculated. Total nitrogen and carbon contents of the samples were determined gas-chromatographically by a Vario MAX CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

Total leaf pool size of immediate isoprene precursor, DMADP, was determined using the acid hydrolysis technique of Fisher et al. (2001) with the modifications of Rasulov et al. (2009a). Differently from the original method (Fisher et al. 2001), isoprene, released during the acid hydrolysis was detected by PTR-MS (Rasulov et al. 2009a). Leaf dry to fresh mass ratio, and leaf dry mass per unit area were used to convert the DMADP contents from leaf fresh mass basis to leaf area basis.

Meteorological characteristics

Temperature measurements recorded by the weather station of the Laboratory of Environmental Physics, University of Tartu (http://meteo.physic.ut.ee) located at 58.37°N, 26.73°E (elevation 76 m), 2.9 km from the study site were employed to determine daily minimum, maximum and mean air temperatures. To detect the effect of past temperature signal on foliage functioning, we calculated average minimum, maximum and mean temperatures for varying number of days, 0 (day of sampling) to 10, preceding foliage sampling.

Data analyses

Linear and non-linear regression techniques in the form of $y = ax^b$ and $y = a\log(x) + b$ were used to test for the correlations among the structural, chemical and physiological characteristics throughout leaf senescence, and with current and past temperature environment. As there was a rapid reduction of air temperatures from ca. 15–20°C to 7–8°C on September 11 with minimum temperatures as low as 4–5°C, and these low temperatures stayed until September 19 (Fig. 1), fitting of the data was conducted in three different ways to test for the robustness of the fitting with and without influential observations and the effect of the stress period: (1) with all data pooled; (2) the data for the stress period (September 11–19) removed; (3) both the late summer measurements and the stress period data removed. The means among different dates were separated by ANOVA followed by Tukey test. We used Origin 8.1 (OriginLab, Northampton, MA, USA) for statistical analyses, and considered all statistical tests significant at $P < 0.05$.

Results

Variation in foliage chemistry and structure through leaf senescence

Foliage N content per area (N_A) decreased slowly from the late summer to the end of September from a value of 2.01 to 1.69 g m^{-2}, i.e. only ca. 16% during this period, corresponding to a rate of decrease of 0.45% day^{-1} (Fig. 2a). After this slow rate of reduction, N_A decreased rapidly to values of ca. 1.3 g m^{-2} on October 6, i.e. a further reduction of ca. 23%, corresponding to the rate of reduction of 3.1% day^{-1} (Fig. 2a). Analogous changes were observed in N content per dry mass (data not shown). Leaf dry mass per unit area (M_A), also decreased during leaf senescence from a value of 88.8 ± 0.5 g m^{-2} in late summer to 76.8 ± 0.4 g m^{-2} during leaf fall, corresponding to the relative reduction of 14% during the whole period of senescence. There was no clear age-dependent variation in leaf dry to fresh mass ratio, and foliage carbon content per dry mass. As the result of invariable carbon content per mass, foliage C/N mass ratio increased from 20.4 in late summer to 31.0 in falling leaves, i.e., ca. 50% increase.

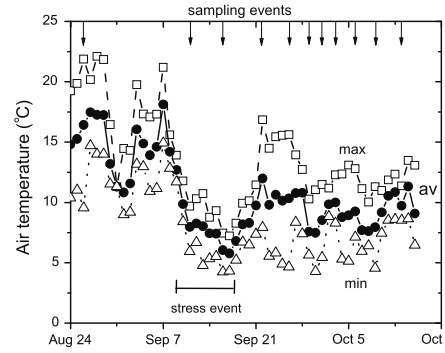

Fig. 1 Variation in daily minimum, maximum and average air temperature at Tartu, Estonia (58.37°N, 26.73°E) during late summer and autumn 2008 (derived from the data of the Laboratory of Environmental Physics, Institute of Physics, University of Tartu, http://meteo.physic.ut.ee/ for raw data). On September 11, average air temperature decreased rapidly from ca. 15–20°C to 7–8°C, and minimum temperatures stayed at 4–5°C until September 19 (chilling stress period)

Foliage photosynthetic activity during senescence

Differently from N_A, leaf net assimilation rate (A) decreased rapidly from 8.6 ± 0.6 μmol m^{-2} s^{-1} in late summer to 3.60 ± 0.27 μmol m^{-2} s^{-1} in early stages of senescence (Fig. 2b) in response to the rapid reductions in temperature in September 11 (Fig. 1). After this rapid reduction of ca 60%, there was initially some recovery, and thereafter, a continuous reduction of net assimilation rate until leaf fall to values as low as 1.3 μmol m^{-2} s^{-1}, with overall reduction from late summer to leaf fall of 85% relative to the initial value (Fig. 2b).

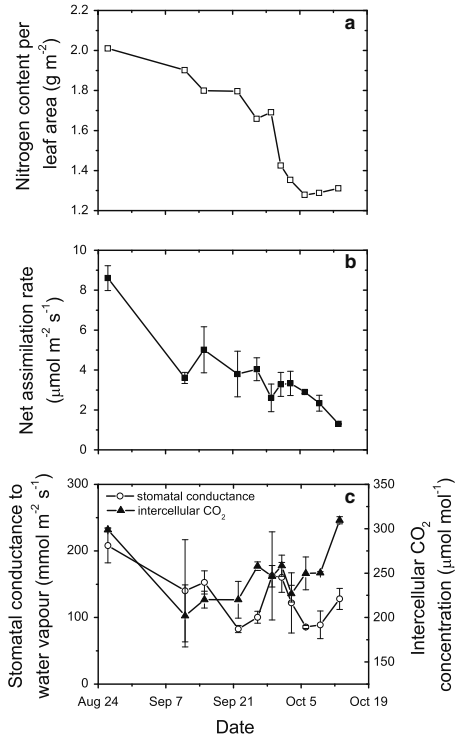

Fig. 2 Time-dependent variation in leaf nitrogen content per area (**a**), net assimilation rate (**b**), and stomatal conductance to water vapour and intercellular CO_2 concentration (**c**) from late summer to leaf fall in temperate deciduous tree *Populus tremula*. Data are averages ± SE of three independent samples. Intensive leaf yellowing and leaf fall started in October 15 (Fig. 1 for temperature during the study period). Foliage gas-exchange measurements were conducted at ambient CO_2 concentration of 380 ± 20 μmol mol^{-1}, leaf temperature of 30 ± 1°C and incident quantum flux density of 600 ± 100 μmol m^{-2} s^{-1}

Analogous changes were observed in stomatal conductance (g_s), especially after the rapid temperature drop (cf. Figs. 1, 2c). After the initial strong reduction, g_s recovered between September 21 and October 1, and again declined thereafter (Fig. 2c). The stress-dependent reduction in g_s between September 11 and 19, also led to reduced concentration of CO_2 in intercellular air space between 100 and 150 μmol mol^{-1} (C_i, Fig. 2c), indicating that the rapid reduction of A after the cold stress was partly due to reduced stomatal conductance ($A = g_s(C_a - C_i)$, where C_a is the ambient CO_2 concentration). However with further progression of leaf senescence, from September 21 onwards, C_i increased to more than 300 μmol mol^{-1} at the last phase of senescence, indicating that A decreased relatively more than g_s.

Isoprene and methanol emission rates, DMADP pool size and the fraction of carbon going into isoprene during leaf aging

Analogously with the rate of photosynthesis, isoprene emission rate decreased sharply during the cold stress episode between September 11 and 19, by ca. 70% relative to the initial value in late summer (Fig. 3a). However, after the cold stress event, isoprene emission rate recovered by 50% by September 26, relative to the observed minimum. Thereafter, isoprene emission rate decreased again with minor recovery in early October, with overall reduction of ca. 80% relative to the late summer estimate (Fig. 3a). Changes in the total leaf pool size of immediate isoprene precursor, DMADP, followed the isoprene emission rate (Fig. 3a). However, the transient recovery of DMADP after the cold stress occurred with a time-shift of ca. 3–5 days compared with the recovery in isoprene emission rates. The overall reduction of ca. 50% during the study period was much less than in the case of isoprene emission. The fraction of photosynthetic carbon lost due to isoprene emission varied between 0.4 and 1%, being higher right after the recovery from the cold stress and also at the end of the study period (Fig. 3b).

Methanol emission occurred at a low level of 0.6–1.2 nmol m^{-2} s^{-1} throughout the study period until the last sampling event when the emission was amplified by almost an order of magnitude (Fig. 3c).

Correlations between foliage chemistry and physiological traits throughout senescence

As the result of simultaneous reduction of foliage physiological activity and nitrogen content, both the net assimilation (Fig. 4a) and isoprene emission rate (Fig. 4b) were positively correlated with nitrogen content. The relationships were stronger with all data pooled than without the late-summer measurements (Fig. 4). As N content decreased initially slowly (Fig. 2a), but both the rates of net assimilation (Fig. 2b), and isoprene emission (Fig. 3a) decreased rapidly after the cold stress event in September 11–19, the slopes of net assimilation and isoprene emission rate versus nitrogen content were shallower without the late summer, non-stressed estimates (Fig. 4).

Isoprene emission rate and net assimilation rate were strongly correlated in all cases (Fig. 5a). In addition, isoprene emission rate was positively correlated with the DMADP pool size (Fig. 5b). Isoprene emission rate and the fraction of carbon going into isoprene emission were not correlated ($r^2 = 0.03$, $P > 0.6$).

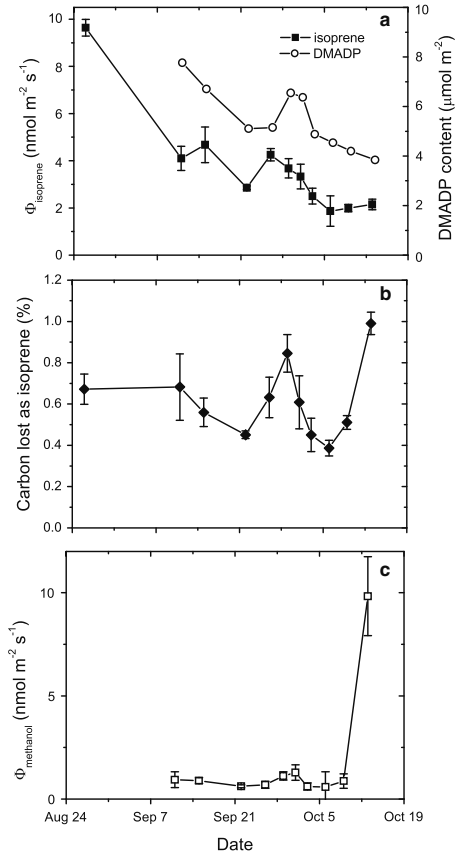

Fig. 3 Variation in the pool size of immediate isoprene precursor, DMADP and leaf isoprene emission rate ($\Phi_{isoprene}$, **a**), the percentage of assimilated carbon lost as isoprene (f_I, **b**), and leaf methanol emission rate ($\Phi_{methanol}$, **c**) in *P. tremula* from late summer to leaf fall. Reported are averages ± SE of three independent measurements. Environmental characteristics in leaf chamber during isoprene and methanol emission measurements as in Fig. 2. As six carbon atoms are lost during formation of isoprene (e.g., Niinemets et al. 1999), the percentage of carbon lost as isoprene is given as $(6\Phi_{isoprene}/A)\cdot 100$, where A is the net assimilation rate

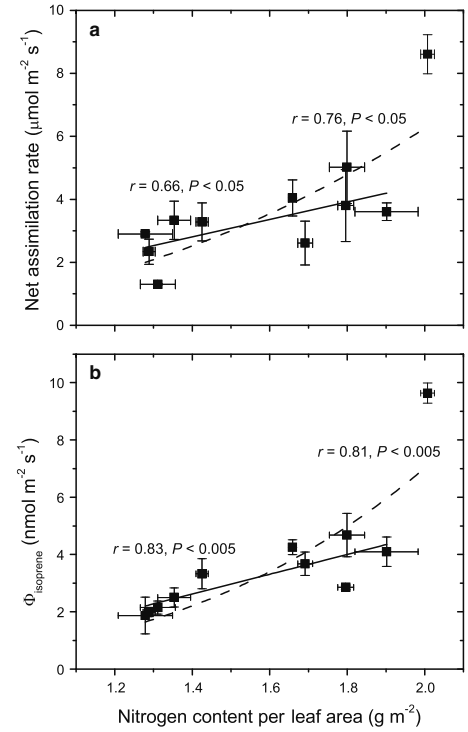

Fig. 4 Dependencies of isoprene emission (**a**) and net assimilation (**b**) rates on leaf nitrogen content in *P. tremula* from late summer through leaf senescence. Data were fitted by either non-linear regressions in the form of $y = ax^b$ (all data pooled) or by linear regressions (fitting without the late-summer measurements). Data in **a** were separately fitted using all data pooled (*dashed line*) and with the late summer estimate removed (*solid line*). *Error bars* show ±SE

Effects of present and past temperatures on isoprene and methanol emissions

Isoprene emission rate was positively correlated with average air temperature, whereas the strength of this relationship depended on the data included and the number of preceding days employed for average temperature calculation (Fig. 6). In the case of all data pooled, the relationship was relatively weak (Fig. 6a), and the highest explained variance was observed with average temperature of 10 days preceding sampling (Fig. 6b). In contrast, significantly larger degree of explained variance was observed when the data corresponding to cold stress event between September 11 and 19 were not included in the analysis (Fig. 6a). In this case, the highest degree of explained variance was observed with 6 days average temperature (Fig. 6b). Analogously, when only the data after the stress period were used, 6-days average temperature also provided the best explanatory power. In addition, in the latter case, the maximum in the explanatory power was sharp with average T for less than 3 days and more than 8 days being statistically non-significant (Fig. 6b).

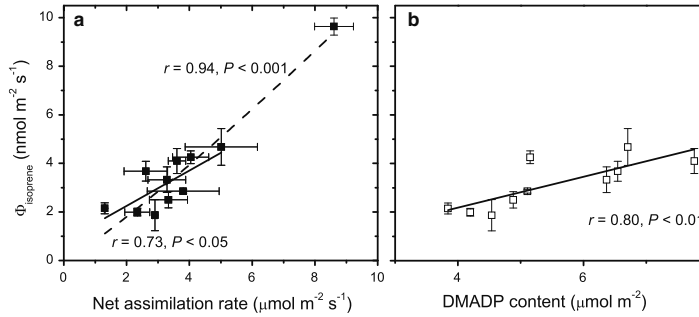

Fig. 5 Relationships of the isoprene emission rate with net assimilation rate (**a**) and with isoprene precursor, DMADP pool size (**b**) from late summer through leaf senescence in *P. tremula*. In **b**, DMADP pool size measurements were not available for late summer. Data presentation as in Fig. 4

The relationships with daily minimum and maximum temperatures were similar to those with the average T, but the maximum explained variance was always less, e.g., for the dataset without the stress period, maximum $r^2 = 0.79$ for daily minimum, and $r^2 = 0.90$ for daily maximum T, while the maximum r^2 was 0.98 for the average temperature (Fig. 6). In the case of methanol emissions, no statistically significant correlations with temperature environment were found ($P > 0.06$).

Discussion

Leaf nitrogen and carbon contents and physiological activity through senescence

We observed classic reductions in foliage N content per area and in leaf dry mass per unit area with altogether ca. 40% resorption of foliar N and 14% resorption of total leaf dry mass, confirming other observations that N is preferably resorbed due to protein degradation, while most of the structural carbon is lost by leaf abscission (e.g., Chapin and Moilanen 1991; Killingbeck 1996; Niinemets and Tamm 2005). Biphasic kinetics in N resorption was observed (Fig. 2a) in agreement with past studies indicating that leaf N resorption proceeds with a relatively slow rate until the final stages of leaf senescence (Grassi et al. 2005; Niinemets and Tamm 2005).

Differently from N, foliage net assimilation rate under standardized conditions of 30°C and incident quantum flux density of 600 μmol m^{-2} s^{-1} (A) decreased rapidly in the initial phase of senescence (Fig. 2b), reflecting the rapid reductions in air temperature, the cold stress event (Fig. 1). Such a rapid reduction of photosynthesis rate is in agreement with previous studies indicating major decreases in photosynthesis after chilling events (Day et al. 1989; DeLucia 1986; DeLucia and Smith 1987; Falge et al. 1996; Gray et al. 1996; Krol et al. 1995). These past studies have demonstrated that both stomatal and non-stomatal factors, in particular, photoinhibition can be responsible for the reduction in photosynthesis during chilling stress. In our study, the rapid reduction in A was paralleled by decreases in stomatal conductance and intercellular CO_2 concentration (C_i, Fig. 2c), suggesting that the reduction in A was at least partly stomatal. At any rate, relatively high values of leaf N content at that time period (cf. Fig. 2a, b), suggest that the rapid reduction in A was not driven by decreases in rate-limiting photosynthetic proteins. In contrast, with further advancement of senescence, C_i actually increased, demonstrating that A was reduced more than g_s, reflecting the rapid N resorption at final stages of senescence (Fig. 2).

Because a large proportion of total leaf N, 25–75%, is invested in proteins and pigments responsible for photosynthesis, strong relationships between leaf N content and foliage photosynthetic capacity are commonly found (Evans 1989 for a review). The positive correlation between N content and A is maintained through leaf senescence (Niinemets et al. 2004; Reich et al. 1991), but the slope of this relationship may change due to modifications in the fractional N investment in photosynthetic machinery (Niinemets and Tenhunen 1997), and as our study demonstrates (Fig. 2), due to inhibition of photosynthesis by cold stress. Although N content is often employed as a convenient substitute of assimilation capacity, this change in the responsiveness of photosynthesis to variations in N content in senescent leaves (Fig. 4a) highlights the danger of predicting foliage photosynthesis from variations in N content during senescence.

Isoprene and methanol emissions in senescing leaves and their relationship to photosynthesis and leaf chemistry

Similarly to photosynthesis, isoprene emission rate under standardized temperature and light conditions decreased rapidly in response to the cold stress event (Fig. 3a). In fact, the reduction in isoprene emission in response to the stress event was even larger than in photosynthesis as

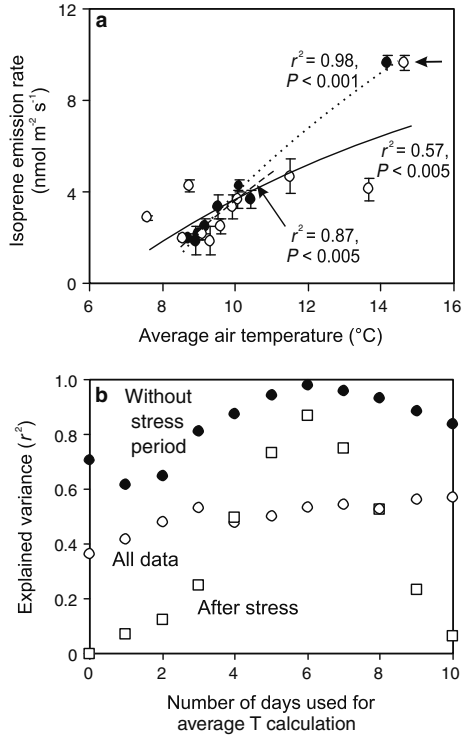

Fig. 6 Correlations of isoprene emission rate with average temperature preceding the measurements (**a**) and the explained variance (r^2) of isoprene emission versus average temperature relationships in dependence on the number of preceding days used for calculation of the average temperature (**b**) in *P. tremula*. Data fitting was conducted with all data pooled (*open circles* in **a** and **b**, *solid line* in **a**), with the data corresponding to stress period (three sampling occasions between September 11–22, s. Fig. 1) removed (*filled circles* in **a** and **b**, *dotted line* in **a**), and with only the data after stress (data corresponding to the stress period and late summer measurements removed, *dashed line* in **a**, *open squares* in **b**), Data were fitted by non-linear regressions in the form of $y = a\log(x) + b$. In **a**, the average temperature giving the largest degree of explained variance (**b**) was used. For all data pooled, this was the average for 10 days preceding the measurements, while for the other two datasets this was the average temperature for the preceding 6 days

suggested by reductions in the fraction of carbon going into isoprene synthesis (Fig. 3b). Such cold stress effects on isoprene emission have been postulated in some studies to explain reductions in isoprene emissions following cold nights (Geron et al. 2000), but have so far not been studied extensively.

After the cold stress, isoprene emissions increased again and slowly decreased through leaf senescence (Fig. 3a) in agreement with previous studies looking at leaf aging effects on isoprene emissions (Harley et al. 1994; Mayrhofer et al. 2005; Monson et al. 1994). The recovery was associated with the increase in the pool size of immediate isoprene precursor, DMADP (Fig. 3a) and also with increases in the fraction of carbon going into isoprene synthesis (Fig. 3b).

As with photosynthesis, there was a significant relationship of isoprene emission with foliar N content (Fig. 4b), but the relationship had a relatively shallow slope in senescing leaves, indicating that the kinetics of the time-dependent reductions of isoprene emission was different from that of bulk leaf N. Isoprene emission rate was also correlated with the net assimilation rate as reported in a number of other studies (Harley et al. 1996; Litvak et al. 1996; Possell et al. 2004), and the relationship was similar for all data pooled and without the late-summer measurements (Fig. 5a). However, the relationship was scattered, reflecting variation in the fraction of carbon going into isoprene emission (Fig. 3b).

Isoprene is synthesized in chloroplasts from DMADP by isoprene synthase enzyme, and ultimately the bulk of the carbon used for isoprene synthesis comes directly from photosynthesis (Lichtenthaler 1999; Logan et al. 2000; Silver and Fall 1991). However, as the fraction of carbon going into isoprene emission in emitting species is generally relatively small, 0.5–2% under non-stressed conditions (e.g., Harley et al. 1994; Niinemets et al. 2010b), photosynthetic rate does not exert a direct control over isoprene emission (Monson and Fall 1989; Niinemets et al. 1999). Nevertheless, a control at the level of energetic equivalents, in particular ATP, controlled by the rate of photosynthetic electron transport, is plausible (Loreto and Sharkey 1993; Niinemets et al. 1999; Rasulov et al. 2010; 2009b). During the senescence, the components of photosynthetic electron transport chain are degraded, inevitably resulting in reduction of ATP level (Keskitalo et al. 2005), and thus, in reduced energy supply for isoprene synthesis. Furthermore, chloroplasts themselves are degraded (Keskitalo et al. 2005), leading to reduction of sites with functionally active isoprene synthase. In our study, the variations in DMADP pool size occurred simultaneously with isoprene emission (Fig. 3a) and the emissions were strongly correlated with DMADP pool size (Fig. 5b). Such variations in DMADP pool size may reflect the temporal variations in ATP status of the leaves, in particular, the reduction during the cold stress event. These data collectively suggest that changes in isoprene emission rate in senescent leaves were driven both by changes in isoprene synthase content and availability of intermediates for isoprene synthesis.

Enhancement of the fraction of carbon going into isoprene emission at the end of the growing season (Fig. 3b) indicates that at the final sampling, photosynthetic activity had been reduced to a greater degree than isoprene emission. Preservation of isoprene emission longer into the senescence is in accordance with the hypothesis that maintenance of isoprene emission helps to protect the plants against the oxidative stress that can become particularly significant during degradation of cellular components (s. Introduction).

Methanol release as the result of activation of pectin methylesterases is a common phenomenon during oxidative stress (Micheli 2001). In our study, methanol emissions occurred at low level of ca. 1 nmol m^{-2} s^{-1} until the last stages of leaf senescence (Fig. 3c), suggesting similar degree of oxidative stress throughout the senescence. Large burst of methanol emission at the final stage of leaf senescence has to our knowledge not been reported before. It may reflect the onset of degradation of cell walls after elicitation of leaf abscission. Previously, important methanol release has been observed from ripening fruits (Frenkel et al. 1998; Wakabayashi et al. 2000).

Environmental controls over isoprene emission during senescence

As past studies have demonstrated (s. Introduction), thermal history exerts an important control on the capacity of isoprene emission, E_S. According to previous studies, E_S in mid-season in fully-developed non-senescent leaves acclimates rapidly to preceding environmental conditions. In *Populus*, isoprene emissions were already enhanced 3 h after transfer to a higher temperature (Wiberley et al. 2008), and important diurnal variations in E_S occur throughout the day tracking the diurnal variations in temperature (Funk et al. 2003; Loivamäki et al. 2007; Wiberley et al. 2009). Analysing longer-term kinetics, Funk et al. (2003) obtained the best explanatory power in E_S versus temperature relationships with the average temperature of 12 h preceding the measurements. Similar rapid changes in E_S have been observed in other species. In *Quercus alba*, the preceding average temperature of past 2 days was found to be the best predictor of modifications in E_S in Sharkey et al. (1999), while 6–18 h was the strongest predictor in Geron et al. (2000). In *Q. alba* and *Q. rubra*, the acclimation occurred within a day (Hanson and Sharkey 2001). Analogously, the variation in monoterpene emission capacity was best explained by average temperature of 2 days preceding the measurements in the constitutive monoterpene emitter *Quercus ilex* (Blanch et al. 2011).

These previous estimates are strongly driven by mid-season data for fully mature non-senescent leaves. In our study, we observed overall low level of explanatory power for the temperature models when all data were pooled (Fig. 6a), with the best explanatory power achieved with average temperature of 10 days preceding the measurements (Fig. 6b). This low explanatory power for all data pooled was associated with the cold stress event (Fig. 3a), during which the emissions decreased sharply. When the data corresponding to this event were left out, the explained variance increased significantly and the average temperature of the 6 preceding days explained the largest proportion of variance (Fig. 6). Compared with previous studies conducted mainly with non-senescent leaves where past temperatures of 6–48 h determined the E_S value, our result demonstrates that the responsiveness of E_S to past temperature is much less in senescing leaves. This result indicates that although E_S does respond to temperature fluctuations in senescing leaves, senescence-driven degradation processes are predominant. Although we hypothesized that the minimum temperature may be more strongly associated with E_S than the average temperature, this hypothesis was not supported by available data, and average temperature was confirmed to be the best predictor of fluctuations in E_S.

So far, isoprenoid emission models assume a constant responsiveness of E_S to past temperature (Grote et al. 2010; Guenther et al. 2006; Lehning et al. 2001). Our study demonstrates that for an important period in temperate environments, the onset of senescence until the leaf fall, the responsiveness of E_S to past temperature is much less than previously estimated and used in the models. Furthermore, a simple temperature relationship assumes that one single process, variation in isoprene synthase activity, is responsible for temperature-driven differences in E_S. Yet, this simple relationship failed to consider the cold stress effects on isoprene emission that were likely driven by sustained modifications in substrate pool size, likely due to reduced level of energetic and reductive co-factors ATP and NADPH in the stressed leaves. To more effectively parameterize the isoprene emission models at the end of the growing season, further work is clearly needed to gain insight into regulation of isoprene emission under low temperature stress.

Overall, this study demonstrates that the temperature-responsiveness of isoprene emission capacity is responsive to fluctuations in temperature also in senescing leaves, but the responsiveness is reduced compared to mid-season. As the period of leaf senescence in deciduous trees covers a significant fraction of the year in temperate ecosystems, this study calls for more advanced consideration of temperature acclimation of isoprene emission capacity during the season in the models.

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References

Adams WW III, Winter K, Schreiber U, Schramel P (1990) Photosynthesis and chlorophyll fluorescence characteristics in relationship to changes in pigment and element composition of leaves of *Platanus occidentalis* L. during autumnal leaf senescence. Plant Physiol 93:1184–1190

Affek HP, Yakir D (2002) Protection by isoprene against singlet oxygen in leaves. Plant Physiol 129:269–277

Andersson A, Keskitalo J, Sjödin A, Bhalerao R, Sterky F, Wissel K, Tandre K, Aspeborg H, Moyle R, Ohmiya Y, Bhalerao R, Brunner A, Gustafsson P, Karlsson J, Lundeberg J, Nilsson O, Sandberg G, Strauss S, Sundberg B, Uhlen M, Jansson S, Nilsson P (2004) A transcriptional timetable of autumn senescence. Genome Biol 5:R24

Arneth A, Schurgers G, Hickler T, Miller PA (2008) Effects of species composition, land surface cover, CO_2 concentration and climate on isoprene emissions from European forests. Plant Biol 10:150–152

Beauchamp J, Wisthaler A, Hansel A, Kleist E, Miebach M, Niinemets Ü, Schurr U, Wildt J (2005) Ozone induced emissions of biogenic VOC from tobacco: relations between ozone uptake and emission of LOX products. Plant Cell Environ 28:1334–1343

Blanch J-S, Llusià J, Niinemets Ü, Noe SM, Peñuelas J (2011) Instantaneous and historical temperature effects on α-pinene emissions in *Pinus halepensis* and *Quercus ilex*. J Environ Biol 32:1–6

Chapin FS III, Moilanen L (1991) Nutritional controls over nitrogen and phosphorus resorption from Alaskan birch leaves. Ecology 72:709–715

Cojocariu C, Solomon SJ, Burrows J, Hewitt CN (2006) Effect of ozone fumigation on isoprene and methanol emission by the leaves of grey poplar (*Populus x canescens*). Geophys Res Abstr 8:03615

Copolovici L, Niinemets Ü (2010) Flooding induced emissions of volatile signalling compounds in three tree species with differing waterlogging tolerance. Plant Cell Environ 33:1582–1594

Day TA, DeLucia EH, Smith WK (1989) Influence of cold soil and snowcover on photosynthesis and leaf conductance in two Rocky Mountain conifers. Oecologia 80:546–552

DeLucia EH (1986) Effect of low root temperature on net photosynthesis, stomatal conductance and carbohydrate concentration in Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) seedlings. Tree Physiol 2:143–154

DeLucia EH (1987) The effect of freezing nights on photosynthesis, stomatal conductance, and internal CO_2 concentration in seedlings of Engelmann spruce (*Picea engelmannii* Parry). Plant Cell Environ 10:333–338

DeLucia EH, Smith WK (1987) Air and soil temperature limitations on photosynthesis in Engelmann spruce during summer. Can J For Res 17:527–533

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78:9–19

Falge E, Graber W, Siegwolf R, Tenhunen JD (1996) A model of the gas exchange response of *Picea abies* to habitat conditions. Trees 10:277–287

Feucht W, Treutter D, Christ E (1997) Role of flavanols in yellowing beech trees of the Black Forest. Tree Physiol 17:335–340

Fisher AJ, Rosenstiel TN, Shirk MC, Fall R (2001) Nonradioactive assay for cellular dimethylallyl diphosphate. Anal Biochem 292:272–279

Fracheboud Y, Luquez V, Björkén L, Sjödin A, Tuominen H, Jansson S (2009) The control of autumn senescence in European aspen. Plant Physiol 149:1982–1991

Frenkel C, Peters JS, Tieman DM, Tiznado ME, Handa AK (1998) Pectin methylesterase regulates methanol and ethanol accumulation in ripening tomato (*Lycopersicon esculentum*) fruit. J Biol Chem 273:4293–4295

Funk JL, Jones CG, Baker CJ, Fuller HM, Giardina CP, Lerdau MT (2003) Diurnal variation in the basal emission rate of isoprene. Ecol Appl 13:269–278

Garcia-Plazaola JI, Hernández A, Becerill JM (2003) Antioxidant and pigment composition during autumnal leaf senescence in woody deciduous species differing in their ecological traits. Plant Biol 5:557–566

Geron C, Guenther A, Sharkey T, Arnts RR (2000) Temporal variability in basal isoprene emission factor. Tree Physiol 20:799–805

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 Physiol 25:349–360

Gray GR, Savitch LV, Ivanov AG, Huner NPA (1996) Photosystem II excitation pressure and development of resistance to photoinhibition. II. Adjustment of photosynthetic capacity in winter wheat and winter rye. Plant Physiol 110:61–71

Grote R, Keenan T, Lavoir A-V, Staudt M (2010) Process-based simulation of seasonality and drought stress in monoterpene emission models. Biogeosciences 7:257–274

Guenther A, Karl T, Harley P, Wiedinmyer C, Palmer PI, Geron C (2006) Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature). Atmos Chem Phys 6:3181–3210

Hansel A, Jordan A, Holzinger R, Prazeller P, Vogel W, Lindinger W (1995) Proton transfer reaction mass spectrometry: on-line trace gas analysis at the ppb level. Int J Mass Spectrom Ion Process 149–150:609–619

Hanson DT, Sharkey TD (2001) Rate of acclimation of the capacity for isoprene emission in response to light and temperature. Plant Cell Environ 24:937–946

Harley PC, Litvak ME, Sharkey TD, Monson RK (1994) Isoprene emission from velvet bean leaves. Interactions among nitrogen availability, growth photon flux density, and leaf development. Plant Physiol 105:279–285

Harley P, Guenther A, Zimmerman P (1996) Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (*Liquidambar styraciflua*) leaves. Tree Physiol 16:25–32

Herrick JD, Thomas RB (2003) Leaf senescence and late-season net photosynthesis of sun and shade leaves of overstory sweetgum (*Liquidambar styraciflua*) grown in elevated and ambient carbon dioxide concentrations. Tree Physiol 23:109–118

Hoch WA, Zeldin EL, McCown BH (2001) Physiological significance of anthocyanins during autumnal leaf senescence. Tree Physiol 21:1–8

Hopkins M, Taylor C, Liu Z, Ma F, McNamara L, Wang TW, Thompson JE (2007) Regulation and execution of molecular disassembly and catabolism during senescence. New Phytol 175:201–214

Ibrahim MA, Mäenpää M, Hassinen V, Kontunen-Soppela S, Malec L, Rousi M, Pietikäinen L, Tervahauta A, Kärenlampi S, Holopainen JK, Oksanen EJ (2010) Elevation of night-time temperature increases terpenoid emissions from *Betula pendula* and *Populus tremula*. J Exp Bot 61:1583–1595

Keskitalo J, Bergquist G, Gardeström P, Jansson S (2005) A cellular timetable of autumn senescence. Plant Physiol 139:1635–1648

Killingbeck KT (1996) Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. Ecology 77:1716–1727

Kontunen-Soppela S, Parviainen J, Ruhanen H, Brosché M, Keinänen M, Thakur RC, Kolehmainen M, Kangasjärvi J, Oksanen E, Karnosky DF, Vapaavuori E (2010) Gene expression responses of paper birch (*Betula papyrifera*) to elevated CO_2 and O_3 during leaf maturation and senescence. Environ Pollut 158:959–968

Krol M, Gray GR, Hurry VM, Öquist G, Malek L, Huner NPA (1995) Low-temperature stress and photoperiod affect an increased tolerance to photoinhibition in *Pinus banksiana* seedlings. Can J Bot 73:1119–1127

Lehning A, Zimmer W, Zimmer I, Schnitzler JP (2001) Modeling of annual variations of oak (*Quercus robur* L.) isoprene synthase activity to predict isoprene emission rates. J Geophys Res 106:3157–3166

Lichtenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annu Rev Plant Physiol Plant Mol Biol 50:47–65

Lindinger W, Hansel A, Jordan A (1998a) On-line monitoring of volatile organic compounds at pptv levels by means of proton-transfer-reaction mass spectrometry (PTR–MS): medical applications, food control and environmental research. Int J Mass Spectrom Ion Process 173:191–241

Lindinger W, Hansel A, Jordan A (1998b) Proton-transfer-reaction mass spectrometry (PTR–MS): on-line monitoring of volatile organic compounds at pptv levels. Chem Soc Rev 27:347–354

Litvak ME, Loreto F, Harley PC, Sharkey TD, Monson RK (1996) The response of isoprene emission rate and photosynthetic rate to photon flux and nitrogen supply in aspen and white oak trees. Plant Cell Environ 19:549–559

Logan BA, Monson RK, Potosnak MJ (2000) Biochemistry and physiology of foliar isoprene production. Trends Plant Sci 5:477–481

Loivamäki M, Louis S, Cinege G, Zimmer I, Fischbach RJ, Schnitzler J-P (2007) Circadian rhythms of isoprene biosynthesis in grey poplar leaves. Plant Physiol 143:540–551

Loreto F, Sharkey TD (1993) On the relationship between isoprene emission and photosynthetic metabolites under different environmental conditions. Planta 189:420–424

Loreto F, Velikova V (2001) Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol 127:1781–1787

Magel E, Mayrhofer S, Müller A, Zimmer I, Hampp R, Schnitzler J-P (2006) Photosynthesis and substrate supply for isoprene biosynthesis in poplar leaves. Atmos Environ 40:S138–S151

Matile P, Schellenberg M, Peisker C (1992) Production and release of a chlorophyll catabolite in isolated senescent chloroplasts. Planta 187:230–235

Mayrhofer S, Teuber M, Zimmer I, Louis S, Fischbach RJ, Schnitzler J-P (2005) Diurnal and seasonal variation of isoprene biosynthesis-related genes in grey poplar leaves. Plant Physiol 139:474–484

Merzlyak MN, Gitelson A (1995) Why and what for the leaves are yellow in autumn? On the interpretation of optical spectra of senescing leaves (*Acer platanoides* L.). J Plant Physiol 145:315–320

Micheli F (2001) Pectin methylesterases: cell wall enzymes with important roles in plant physiology. Trends Plant Sci 6:414–419

Monson RK, Fall R (1989) Isoprene emission from aspen leaves. Influence of environment and relation to photosynthesis and photorespiration. Plant Physiol 90:267–274

Monson RK, Harley PC, Litvak ME, Wildermuth M, Guenther AB, Zimmerman PR, Fall R (1994) Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves. Oecologia 99:260–270

Niinemets Ü, Tamm Ü (2005) Species differences in timing of leaf fall and foliage chemistry modify nutrient resorption efficiency in deciduous temperate forest stands. Tree Physiol 25:1001–1014

Niinemets Ü, Tenhunen JD (1997) A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. Plant Cell Environ 20:845–866

Niinemets Ü, Tenhunen JD, Harley PC, Steinbrecher R (1999) A model of isoprene emission based on energetic requirements for isoprene synthesis and leaf photosynthetic properties for *Liquidambar* and *Quercus*. Plant Cell Environ 22:1319–1336

Niinemets Ü, Kull O, Tenhunen JD (2004) Within canopy variation in the rate of development of photosynthetic capacity is proportional to integrated quantum flux density in temperate deciduous trees. Plant Cell Environ 27:293–313

Niinemets Ü, Arneth A, Kuhn U, Monson RK, Peñuelas J, Staudt M (2010a) The emission factor of volatile isoprenoids: stress, acclimation, and developmental responses. Biogeosciences 7:2203–2223

Niinemets Ü, Copolovici L, Hüve K (2010b) High within-canopy variation in isoprene emission potentials in temperate trees: implications for predicting canopy-scale isoprene fluxes. J Geophys Res Biogeosci 115:G04029

Niinemets Ü, Monson RK, Arneth A, Ciccioli P, Kesselmeier J, Kuhn U, Noe SM, Peñuelas J, Staudt M (2010c) The leaf-level emission factor of volatile isoprenoids: caveats, model algorithms, response shapes and scaling. Biogeosciences 7:1809–1832

Pétron G, Harley P, Greenberg J, Guenther A (2001) Seasonal temperature variations influence isoprene emission. Geophys Res Lett 28:1707–1710

Possell M, Heath J, Hewitt CN, Ayres E, Kerstiens G (2004) Interactive effects of elevated CO_2 and soil fertility on isoprene emissions from *Quercus robur*. Glob Change Biol 10:1835–1843

Procházková D, Wilhelmová N (2007) Leaf senescence and activities of the antioxidant enzymes. Biol Plant 51:401–406

Rakitskaya TL, Truba AS, Raskola LA, Bandurko AY, Golub AA (2006) Effect of the structure of copper(II) complexes, adsorbed on the surface of SiO_2, on their catalytic activity in ozone decomposition. Theor Exp Chem 42:60–66

Rasulov B, Copolovici L, Laisk A, Niinemets Ü (2009a) Postillumination isoprene emission: in vivo measurements of dimethylallyldiphosphate pool size and isoprene synthase kinetics in aspen leaves. Plant Physiol 149:1609–1618

Rasulov B, Hüve K, Välbe M, Laisk A, Niinemets Ü (2009b) Evidence that light, carbon dioxide and oxygen dependencies of leaf isoprene emission are driven by energy status in hybrid aspen. Plant Physiol 151:448–460

Rasulov B, Hüve K, Bichele I, Laisk A, Niinemets Ü (2010) Temperature response of isoprene emission in vivo reflects a combined effect of substrate limitations and isoprene synthase activity: a kinetic analysis. Plant Physiol 154:1558–1570

Rasulov B, Hüve K, Laisk A, Niinemets Ü (2011) Induction of a longer-term component of isoprene release in darkened aspen leaves: origin and regulation under different environmental conditions. Plant Physiol 156 (in press)

Reich PB, Walters MB, Ellsworth DS (1991) Leaf age and season influence the relationships between leaf nitrogen, leaf mass per area and photosynthesis in maple and oak trees. Plant Cell Environ 14:251–259

Rosenthal SI, Camm EL (1997) Photosynthetic decline and pigment loss during autumn foliar senescence in western larch (*Larix occidentalis*). Tree Physiol 17:767–775

Schnitzler JP, Lehning A, Steinbrecher R (1997) Seasonal pattern of isoprene synthase activity in *Quercus robur* leaves and its significance for modeling isoprene emission rates. Bot Acta 110:240–243

Schnitzler JP, Graus M, Kreuzwieser J, Heizmann U, Rennenberg H, Wisthaler A, Hansel A (2004) Contribution of different carbon sources to isoprene biosynthesis in poplar leaves. Plant Physiol 135:152–160

Sharkey TD, Yeh SS (2001) Isoprene emission from plants. Annu Rev Plant Physiol Plant Mol Biol 52:407–436

Sharkey TD, Singsaas EL, Lerdau MT, Geron CD (1999) Weather effects on isoprene emission capacity and applications in emissions algorithms. Ecol Appl 9:1132–1137

Sharkey TD, Wiberley AE, Donohue AR (2008) Isoprene emission from plants: why and how. Ann Bot 101:5–18

Silver GM, Fall R (1991) Enzymatic synthesis of isoprene from dimethylallyl diphosphate in aspen leaf extracts. Plant Physiol 97:1588–1591

Spasova I, Nikolova P, Mehandjiev D (2007) Ozone decomposition over alumina-supported copper, manganese and copper-manganese catalysts. Ozone Sci Eng 29:41–45

Velikova V, Loreto F (2005) On the relationship between isoprene emission and thermotolerance in *Phragmites australis* leaves exposed to high temperatures and during the recovery from a heat stress. Plant Cell Environ 28:318–327

Vickers CE, Gershenzon J, Lerdau MT, Loreto F (2009) A unified mechanism of action for volatile isoprenoids in plant abiotic stress. Nat Chem Biol 5:283–291

von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153:376–387

Vuorinen T, Nerga A-M, Vapaavuori E, Holopainen JK (2005) Emission of volatile organic compounds from two silver birch (*Betula pendula* Roth) clones grown under ambient and elevated CO_2 and different O_3 concentrations. Atmos Environ 39:1185–1197

Wakabayashi K, Chun J-P, Huber DJ (2000) Extensive solubilization and depolymerization of cell wall polysaccharides during avocado (*Persea americana*) ripening involves concerted action of polygalacturonase and pectinmethylesterase. Physiol Plant 108:345–352

Wiberley AE, Linskey AR, Falbel TG, Sharkey TD (2005) Development of the capacity for isoprene emission in kudzu. Plant Cell Environ 28:898–905

Wiberley AE, Donohue AR, Meier ME, Westphal MM, Sharkey TD (2008) Regulation of isoprene emission in *Populus trichocarpa* leaves subjected to changing growth temperature. Plant Cell Environ 31:258–267

Wiberley AE, Donohue AR, Westphal MM, Sharkey TD (2009) Regulation of isoprene emission from poplar leaves throughout a day. Plant Cell Environ 32:939–947

Yoshida S (2003) Molecular regulation of leaf senescence. Curr Opin Plant Biol 6:79–84

Zapata JM, Guéra A, Esteban-Carrasco A, Martín M, Sabater B (2005) Chloroplasts regulate leaf senescence: delayed senescence in transgenic ndhF-defective tobacco. Cell Death Differ 12:1277–1284

Zentgraf U, Hemleben V (2008) Molecular cell biology: are reactive oxygen species regulators of leaf senescence? In: Lüttge U, Beyschlag W, Murata J (eds) Progress in Botany, vol 69, pp 117–138

CURRICULUM VITAE

I General

First name:	Zhihong
Family name:	Sun
Birth Date:	February 3rd 1972, Shandong, China
Institution:	Department of Plant Physiology, Institute of Agricultural and Environmental Sciences,Estonian University of Life Sciences, Kreutzwaldi 5, EE-51014 Tartu, Estonia
Education:	
2008-2013:	Ph.D studies in Plant Physiology, Estonian University Life Sciences
2002-2005:	MSc studies in Pomology, Chinese Academy of Agriculture Science
1993-1997:	BSc studies in Agronomy, Shangdong Agriculture University
1989-1993:	Third Senior high school of Tai'an city, Shandong

II Scientific and research activities

Research interests:

BVOCs Ecophysiology, Interaction of Global climate change and plants resistance and adaptation

Scholarship

2008-2012:	European Social Fund (Doctoral Studies and Internationalization Programme DoRa) scholarship
2012 Feb.	European COST (Coopreation in science and technology) program travel founds for 1st TERRABITES Training school on terrestrial biosphere modelling, in Italy
2010. May:	Doctoral Studies and Internationalization Programme "DoRa" travel found,for conference of BiogenicHydrocarbons & The Atmosphere, in Switzerland

2009 June: Doctoral Studies and Internationalization Programme "DoRa" travel founds, for conference of Goldschmidt™2009 - "Challenges to Our Volatile Planet", in Switzerland

2008. June: Erasmus Lifelong Learning Programme EU funded scheme found, for Training on tools and methods of sustainable rural development in context with global change in European mountains areas, in Spain.

Grants:

--- ETF7645(Ulo Niinemets)Constitutive and induced emission of volatile isoprenoids in forest trees

--- ETF9253 (Ulo Niinemets)Environmental, physiological and genetic controls on isoprene emission in Populus and Salix

--- ETF9089 (Lucian Octav Copolovici) Volatile compounds and secondary metabolites from Betulaceae and Fagaceae during biotic and abiotic stresses

--- SF1090065s07 (Ulo Niinemets)Plant stress ecophysiologyEstonian

--- Centre of Excellence ENVIRON travel found International conference: 2nd TERRABITES Symposium, ESA ESRIN, Frascati, Italy

Membership in Professional Organizations:

2009-2011 Member of the Society for Experimental Biology (SEB)

2012- Reviewer for Chinese Journal of Plant Ecology

Internal course attended:

2008 June Training on tools and methods of sustainable rural development in context with global change in European mountains areas. ERASMUS Intensive programme IP course Sustmont in Spain

2012 Feb. 1st TERRABITES Training school on terrestrial biosphere modelling, in Italy

2011 Aug. Ph.D course: Plant–Atmosphere Interactions in a Changing Climate Sweden, University of Gothenburg

Prizes:

2005 June	Award of the national (Chinese) outstanding master degree thesis
2007 Sep.	Award of Science and Technology second prize (Soilless and off-season cultivation technique in watermelon) from Beijing Daxing District Science and Technology Commission

ELULOOKIRJELDUS

I Üldandmed

Eesnimi: Zhihong

Perekonnanimi: Sun

Sünniaeg: 3. veebruar 1972, Shandong, Hiina

Õppeasutus: Taimefüsioloogia osakond, Põllumajandus- ja keskkonnainstituut, Eesti Maaülikool, Kreutzwaldi 5, EE-51014 Tartu, Estonia

Haridus:

2008-2013: Doktoriõpe, taimefüsioloogia, Eesti Maaülikool

2002-2005: Magistriõpe, pomoloogia, Hiina Põllumajandusakadeemia (*Chinese Academy of Agriculture Science*)

1993-1997: Bakalaureuseõpe, agronoomia, Shangdong'i Põllumajandusülikool (*Shangdong Agriculture University*)

1989-1993: Keskharidus - *Third Senior high school of Tai'an city, Shandong*

II Teadustöö

Teadustöö valdkond:

Bioorgaanilised lenduvühendid (*BVOC*), ökofüsioloogia, globaalsete kliimamuutuste ning taimede vastupanuvõime ja kohastumise vastastikmõju

Stipendiumid ja toetused:

2008-2012: Euroopa Sotsiaalfond (Doktoriõppe ja rahvusvahelistumise programm DoRa) stipendium;

Veebruar, 2012 Programmi COST (Koostöö teaduse ja tehnoloogia alal) *European COST (Coopreation in science and technology*) reisitoetus osalemiseks rahvusvahelisel kursusel "*1st TERRABITES Training school on terrestrial biosphere modelling*" Itaalias;

Mai, 2010 Euroopa Sotsiaalfondi (Doktoriõppe ja rahvusvahelistumise programm DoRa) reisitoetus osalemaks konverentsil "*Biogenic Hydrocarbons & The Atmosphere*" Šveitsis;

Juuni, 2009 Euroopa Sotsiaalfondi (Doktoriõppe ja rahvusvahelistumise programm DoRa) reisitoetus osalemaks konverentsil Goldschmidt™2009 - *"Challenges to Our Volatile Planet"* Šveitsis;

Juuni, 2008 Euroopa elukestva õppe programmi Erasmus toetus osalemiseks rahvsuavheliselt kursusel Hispaanias.

Toetused:

--- ETF7645(Ülo Niinemets) "Konstitutiivne ja indutseeritud lenduvate isoprenoidide emissioon metsapuudel";

--- ETF9253 (Ülo Niinemets) "Isopreeni emissioon papli- ja pajuliikidel: keskkonna-, füsioloogilised ja geneetilised kontrollmehhanismid";

--- ETF9089 (Lucian Octav Copolovici) "Abiootiliste ja biootiliste stressifaktorite all kannatatavate kaseliste ja pöögiliste emissioonid ja mittelenduvate sekundaarsete metaboliitide sisaldused.";

--- SF1090065s07 (Ülo Niinemets) Väikesemahulise teaduse infrastruktuuri kaasajastamine teadusteema SF1090065s07 raames;

---TK 107 (Ülo Niinemets) "Keskkonnamuutustele kohanemise tippkeskus", toetus osalemiseks rahvsuavhelisel kursusel ja sümpoosiumil TERRABITES. ESA ESRIN, Frascati, Itaalia.

Kuulumine erilaorganisatsioonidesse:

2009-2011 Liige, Eksperimentaalbioloogia Selts (*Member of the Society for Experimental Biology - SEB*);

2012- Retsensent, *Chinese Journal of Plant Ecology.*

Osalemine rahvusvahelistel kursustel:

Juuni, 2008 "Kursus kestlikku arengut tagavatest vahenditest ja meetoditest Euroopa mäestike piirkonnas asuvates maapirkondades" (*Training on tools and methods of sustainable rural development in context with global change in European mountains areas*). ERASMUSe intensiivprogramm, IP kursus, Sustmont, Hispaania;

Veebruar, 2012	Esimene TERRABITES kursus maismaa biosfääri modelleerimisest, (*1st TERRABITES Training school on terrestrial biosphere modelling*), Itaalia;
August, 2011	Doktorikursus: "Taim – atmosfääri vastastikmõju muutuvas kliimas" (*Plant–Atmosphere Interactions in a Changing Climate*), Rootsi, Göteborgi Ülikool.

Autasud:

Juuni, 2005	Hiina riiklik autasu väljapaistva magistritöö eest;
September, 2007	Beijing Daxing piirkonna Teaduse ja Tehnoloogia Komitee II autasu arbuusi kultiveerimist käsitleva töö eest (*Soilless and off-season cultivation technique in watermelon*).

Publikatsioonid

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1) **Sun Z**, Hüve K, Vislap V, Niinemets Ü. (2013). Elevated growth [CO_2] enhances isoprene emissions under high temperatures and improves thermal resistance in hybrid aspen. (Esitatud *Global Change Biology*).
2) **Sun Z**, Niinemets Ü, Hüve K, Rasulov B, Noe SM (2013). Elevated atmospheric CO_2 concentration leads to increased whole-plant isoprene emission in hybrid aspen (*Populus tremula* x *P. tremuloides*). *New Phytologist,* 198, 788-800.
3) **Sun Z**, Niinemets Ü, Hüve K, Noe SM, Rasulov B, Copolovici L, Vislap V. (2012) Enhanced isoprene emission capacity and altered light responsiveness in aspen grown under elevated atmospheric CO_2 concentration. *Global Change Biology*, 18, 3423-3440.
4) **Sun Z**, Copolovici L, Niinemets Ü (2012). Can the capacity for isoprene emission acclimate to environmental modifications during autumn senescence in temperate deciduous tree species *Populus tremula*? *Journal of Plant Research,* **125**, 263-274.
5) **Sun Z**, Wei Q, Yang C, Sun ZF, Wang X (2008). Relationships between distribution of shoots, leaves and temperature, relative humidity in the canopy of Red Fuji apple trees. *Journal of Fruit Science* (Chinese journal), **25**, 6-11. Hiina keeles.
6) **Sun Z**, Wei Q, Yang C, Sun ZF, Wang X (2008) Relationships between distribution of relative light intensity and shoots and foliage in different tier of canopy of Red Fuji apple trees with modified open centre shape. *Journal of Fruit Science* (Chinese journal), **25**, 145-150. Hiina keeles.
7) **Sun Z**, Sun ZF, Yang C, Wang Y (2005). Advances on research and application of mathematical simulation in fruit tree ecophysiology. *Journal of Fruit Science* (Chinese journal), **22**, 361-366. Hiina keeles.
8) **Sun Z**, Li Z, Yang C, Yang Q (2007). Effects of the content of organic manure in siolless cultivation on the yield and qualities of watermelon. *China Cucurbits and Vegetables* (Chinese journal), **1**, 7-10. Hiina keeles.

9) Wang Y, Sun ZF, Guo S, An C, Du H, **Sun Z** (2005). Comparison of frost hardiness of maize varieties in Yanbei region. *Chinese Journal of Agrometeorology*, **26**, 233-235. Hiina keeles.

10) Graduate thesis for Master degree: **Sun Z** (2005). Studies on the relationship between the fruit quality and the microclimate in apple tree canopies. Hiina keeles.

Konverentsi teesid:

1) **Sun Z**, Niinemets Ü, Vislap V, Hüve K(2012). Heat stress responsiveness of hybrid aspen (*Populus tremula x P. tremuloides*) grown under elevated CO_2. Plant Abiotic Stress Tolerence II, Program and Abstract, N23.

2) **Sun Z**, Niinemets Ü, Noe S, Rasulov B (2012). The Influence of elevated CO_2 on isoprene Emission on Leaf and Canopy Scale from Aspen (*Populus tremula x P. tremuloides*). 2nd TERRABITES SYMPOSIUM abstract book, 29.

3) **Sun Z**, Niinemets Ü, Hüve K, Noe S, Rasulov B. (2011). Will higher CO_2 concentration influence our atmosphere quality by changing isoprene emission rate from plants in the future? Society for experimental biology annual main meeting (SEB), Abstract book, 204.

4) **Sun Z**, Niinemets Ü, Copolovici L. (2009). Foliar isoprene emission during autumn senescence in aspen (*Populus tremula)*. Goldschmidt Conference Geochimica et Cosmochimica Acta, 73, A1295-A1295.

5) **Sun Z**, Niinemets Ü, Copolovici L. (2009) Foliar methanol and isoprene emission during autumn senescence in aspen (*Populus tremula)*. VOCBAS science conference 2009 induced Bvoc emissions: Processes and feedback.

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Publications:

1) **Sun Z**, Hüve K, Vislap V, Niinemets Ü. (2013). Elevated growth [CO_2] enhances isoprene emissions under high temperatures and improves thermal resistance in hybrid aspen. (Submitted *Global Change Biology*).

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VIIS VIIMAST KAITSMIST

KAIRE TOMING

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LAHUSTUNUD ORGAANILINE AINE JA SELLE ÖKOLOOGILINE TÄHTSUS
SUURTES MADALATES VEEKOGUDES
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AND NITROGEN CYCLES IN EUROPEAN FOREST ECOSYSTEMS
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SEOSED TAIMSE VARISEGA
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