Biosynthesis and emission of isoprenoids of boreal and tropical tree species in response to drought

Thesis submitted in partial fulfilment of the requirements of

the degree Doctor rer. nat. of the

Faculty of Environment and Natural Resources,

Albert-Ludwigs-Universität

Freiburg im Breisgau, Germany

by

Lars Erik Daber

Freiburg im Breisgau, Germany

2023

universität freiburg

Dean of the faculty: Prof. Dr. Heiner Schanz

First supervisor: Prof. Dr. Christiane Werner

Second supervisor: Prof. Dr. Jonathan Williams

Second reviewer: Prof. Dr. Jörg-Peter Schnitzler

Fourth examiner: Prof. Dr. Andreas Christen

Date of thesis defence: 15.11.2023

Summary

Isoprenoids represent the dominant compound class of biogenic volatile organic compounds emitted by plants. Isoprene and monoterpenes have profound effects on atmospheric chemistry and shape the chemical landscape of many ecosystems and plant-environmental interactions therein. The accuracy of global estimates of emission development in regard to a changing climate and increasing drought frequency is limited by our understanding of the fundamental processes driving emission and biosynthesis of isoprenoids by plants under varying physiological conditions. Further, the effect of drought on compounds utilised for above- and below-ground plant-environmental signalling, such as chiral monoterpenes, is so far hard to assess. The mirrored conformation of the two enantiomers of chiral monoterpenes alters their biological activity but not their physical properties, complicating analytical distinction, which is why they are often overlooked in environmental studies.

Accordingly, the overarching scope of my thesis was to further our understanding of the effects of drought on above- and below-ground emission and biosynthesis of isoprenoids. A specific focus lied on the investigation of emission pattern and biosynthetic dynamics of chiral monoterpenes. Within the framework of the unprecedented drought experiment in a tropical rainforest in the Biosphere 2 (during the Biosphere 2 Water and Life Dynamics campaign 2019), an automated leaf gas exchange measurement system was set up to continuously monitor foliar fluxes of H_2O, CO_2 and volatile organic compounds. Stable isotope labelling experiments with position-specifically labelled pyruvate were performed before and during the drought to investigate changes in above- and, for the first time, below-ground carbon utilisation by four representative plant species from different plant functional types. The results of these experiments were explored in three explicit research studies (**Study 1-3**). Within these studies, I demonstrated that plant species of different plant functional groups vary in their metabolic responses to drought and disproportionally affect various ecosystem fluxes. Diminishing atmospheric isoprene concentrations in response to drought were partially driven by the legume tree

C. fairchildiana, which increasingly resorted to cytosolic pyruvate for isoprene emissions, as the supply of fresh photosynthates available for biosynthesis declined.

The findings from these studies were further developed and adapted to analyse above- and below-ground chiral monoterpene emission and biosynthesis by isotopically labelling *Picea abies* saplings during a severe drought climate chamber experiment (**Study 4** and **Study 5**). In **Study 4**, I revealed that three distinct clusters of chiral monoterpenes are synthesised in needles of *Picea abies* by introducing multiple isotopic tracers ($^{13}CO_2$, ^{13}C1-pyruvate, ^{13}C2-pyruvate). Biosynthesis of chiral monoterpenes is supposed to originate from the same precursor pool but seems to be driven by different metabolic pathways. I conclude that biosynthesis of different groups of chiral monoterpenes is compartmented, either between different cell types or within cell compartments.

De novo biosynthesis and emissions of chiral monoterpenes declined in drought-affected plants, even though storage pool size increased. I therefore argue in **Study 5** that emission of chiral monoterpenes from storage compartments is not a solely passive process. Likely, release of monoterpenes by resin ducts and associated cells or transport mechanisms are disrupted by drought, leading to declining emissions. There was no *de novo* synthesis of chiral monoterpenes detected in roots, indicating that below-ground emissions might be supplied by precursors from above-ground tissue. Notably, chiral monoterpene composition was tissue-specific and drought only affected chiral composition in needles, but not in roots. In conclusion, this thesis provides evidence that drought unequally affects above- and below-ground emission and biosynthesis of isoprenoids and implies important findings for atmospheric model parametrisation and our understanding of the effects of a changing climate on plant-environmental interactions.

Zusammenfassung

Die Molekülgruppe der Isoprenoide macht den größten Anteil biogener organischer Verbindungen aus, die global von Pflanzen emittiert werden. Emissionen von Isopren und Monoterpenen haben tiefgreifende Auswirkungen auf die Atmosphärenchemie und formen die chemische Landschaft vieler Ökosysteme sowie deren komplexe Interaktionen zwischen Pflanzen und anderen Organismen. Die Genauigkeit globaler Modelle zur Abschätzung der Effekte des Klimawandels auf biogene Emissionen von Isopren und Monoterpenen, ist hierbei durch unser Verständnis der fundamentalen Prozesse beschränkt, die die Biosynthese und Emission durch Pflanzen unter variierenden physiologischen Zuständen steuern. Des Weiteren sind die Effekte von Dürreperioden auf über- und unterirdische Emissionen von Signalmolekülen, wie zum Beispiel chiralen Monoterpenen, weitgehend unerforscht. Die gespiegelte Konformation der zwei Enantiomere chiraler Monoterpene ändert zwar deren biologische Aktivität, nicht aber die physikalischen Eigenschaften. Dies erschwert die analytische Trennung der Enantiomere, weshalb sie in umweltwissenschaftlichen Studien oft nur zusammengefasst betrachtet werden.

Das übergeordnete Ziel dieser Arbeit war es daher unser Verständnis von regulatorischen Prozessen zu erweitern, die die über- und unterirdische Emission und Biosynthese von Isopren und teils chiralen Monoterpenen in Reaktion auf Trockenstress steuern. Im Zuge des groß angelegten Trockenstress Experimentes (während der „Biosphere 2 Water and Life Dynamics“ Kampagne 2019), wurde ein automatisiertes Gasaustausch-Messsystem in dem experimentellen Regenwald der Biosphere 2 (Arizona, USA) aufgebaut, um die Blattflüsse von H_2O, CO_2 und volatilen organischen Verbindungen zu überwachen. Vor und während der Dürre wurden stabile Isotopenmarker in Blätter und erstmals in Wurzeln von vier repräsentativen Pflanzenarten verschiedener funktioneller Gruppen eingebracht, um Änderungen in der Verwertung verschiedener Kohlenstoffquellen für primäre Stoffwechselwege und die Synthese von Isopren und Monoterpenen zu untersuchen. Die Ergebnisse dieser Experimente wurden in drei verschiedenen wissenschaftlichen Arbeiten untersucht (**Studie 1-3**). Mit meinen Analysen konnte ich zeigen, dass sich die funktionellen Gruppen in ihrem Einfluss auf verschiedene Ökosystemflüsse,

sowie ihren Stoffwechselreaktionen auf den Trockenstress stark unterschieden. Der Einbruch der atmosphärischen Isopren Konzentrationen unter zunehmender Trockenheit wurde unter anderem durch die Leguminose *Clitoria fairchildiana* beeinflusst. Die restlichen Emissionen von Isopren von *C. fairchildiana* wurden zunehmend von cytosolischen Kohlenstoffquellen gespeist als die Photosyntheserate unter dem Trockenstress zurückging.

Die Erkenntnisse aus diesen Studien dienten der Entwicklung weiterführender Isotopenmarker-Experimente, um die Zusammensetzung chiraler Monoterpene in den Emissionen und Speicherorganen von Nadeln und Wurzeln von *Picea abies* Jungbäumen in einem Trockenstress Versuch in Klimakammern zu untersuchen (**Studie 4** und **Studie 5**).

In **Studie 4** habe ich drei distinkte Gruppen innerhalb der emittierten, chiralen Monoterpene, durch das Einbringen verschiedener Isotopenmarker ($^{13}CO_2$, ^{13}C1-Pyruvat, ^{13}C2-Pyruvat), offengelegt. Diese Beobachtung war überraschend, da die Synthese verschiedener Monoterpene prinzipiell auf das gleiche Vorprodukt (Geranylpyrophosphat) zurückzuführen ist. Aus diesen Erkenntnissen schloss ich, dass die Synthese verschiedener, chiraler Monoterpene höchstwahrscheinlich kompartimentiert ist, entweder zwischen den verschiedenen Zelltypen der Nadeln oder innerhalb einzelner Zellen.

Die Emission chiraler Monoterpene und deren *De-novo*-Synthese wurde während der extremen Trockenphase stark reduziert, obwohl sich die Konzentration der gespeicherten chiralen Monoterpene beinahe verdoppelte. Daher argumentiere ich in **Studie 5**, dass der Anteil der Emissionen aus Speicherorganen nicht ausschließlich passiv abläuft, sondern höchstwahrscheinlich regulative Prozesse die Freisetzung der volatilen Stoffe aus den Harzkanälen, oder andere Transportprozesse diese Emissionen beeinflussen und unter Trockenheit unterbrechen. Innerhalb der Wurzeln konnte keine *De-novo*-Synthese chiraler Monoterpene detektiert werden, was vermuten lässt, dass die unterirdische Synthese dieser Stoffe durch den Phloemtransport komplexerer Vorprodukte über den Stamm versorgt wird. Interessanterweise unterschied sich die Zusammensetzung der chiralen Monoterpene stark zwischen Nadeln und Wurzeln. Mit zunehmender Trockenheit veränderte sich die chirale Zusammensetzung in den Emissionen und Speicherorganen

von Nadeln, blieb jedoch unverändert in den Wurzeln, was vermutlich durch organspezifische Funktionen der chiralen Monoterpene zu erklären ist.

Die gesammelten Erkenntnisse dieser Arbeit liefern Nachweise dafür, dass sich Trockenheit unterschiedlich auf die über- und unterirdische Emission und Biosynthese von Isoprenoiden auswirkt. Die durchgeführten Studien implizieren wichtige Ergebnisse für die Parametrisierung globaler Emissionsmodelle sowie für unser Verständnis der Effekte des Klimawandels auf Interaktionen von Pflanzen mit ihrer Umwelt.

Table of contents

Summary i

Zusammenfassung iii

List of figures ix

Abbreviations x

1. Introduction 1

1.1. Volatile organic compounds in a changing climate 1

1.2. Functions, emission and biosynthesis of isoprenoids 2

1.3. Chiral monoterpenes 7

1.4. Scope of this thesis 9

1.5. Experimental design and approaches 10

1.6. Research objectives and hypotheses 15

2. Main results and discussion 19

2.1. The Biosphere 2 Water and Life Dynamics (B2WALD) drought experiment 19

2.1.1. Ecosystem fluxes are disproportionally affected by different plant functional groups 19

2.1.2. Differences in label incorporation into isoprene and monoterpenes in a tropical legume tree in response to drought 21

2.2. Position-specific pyruvate label indicates significant *de novo* synthesis of chiral monoterpenes in needles but not in roots of Norway spruce 23

2.3. Above- and below-ground differences in chiral monoterpene composition imply information on tissue-specific functioning 26

2.4. Chiral monoterpene emissions are regulated independent of storage pool size 27

3. Conclusion and outlook ____ 29

3.1. Work in progress and future directions ____ 29

3.2. Conclusions ____ 31

4. References ____ 33

5. Study overview and specific contribution ____ 55

Appendix: Cumulative studies ____ 59

Study 1 ____ 59

Ecosystem fluxes during drought and recovery in an experimental forest ____ 59

Study 2 ____ 61

Elucidating Drought-Tolerance Mechanisms in Plant Roots through ^{1}H NMR Metabolomics in Parallel with MALDI-MS, and NanoSIMS Imaging Techniques ____ 61

Study 3 ____ 63

Leaf-level metabolic changes in response to drought affect daytime CO_2 emission and isoprenoid synthesis ____ 63

Study 4 ____ 65

Position-specific isotope labelling gives new insights into chiral monoterpene synthesis of Norway spruce (*Picea abies* L.) ____ 65

Study 5 ____ 67

Chiral monoterpene dynamics of shoots and roots of Norway spruce in response to drought ____ 67

Statutory declaration ____ 69

Acknowledgements ____ 70

List of figures

Figure 1 Schematic representation of atmospheric processes affected by volatile organic compounds from biogenic and anthropogenic sources. _____2

Figure 2 Metabolic pathways leading to the biosynthesis of isoprenoids in plant cells. _____6

Abbreviations

B2	Biosphere 2
B2WALD	Biosphere 2 Water and Life Dynamics
BVOC	Biogenic volatile organic compound
DMADP	Dimethylallyl diphosphate
EI	Electron ionisation
ET	Evapotranspiration
GC-MS	Gas-chromatography mass spectrometer
CI	Chemical ionisation
IDP	Isopentenyl diphosphate
IDPS	Isopentenyl diphosphate synthase
IRGA	Infrared gas analyser
IRIS	Isotope ratio infrared spectrometer
IRMS	Isotope ratio mass spectrometer
IspS	Isoprene synthase
FDP	Farnesyl diphosphate
FDPS	Farnesyl diphosphate synthase
GA-3-P	Glyceraldehyde 3-phosphate
GDP	Geranyl diphosphate
GDPS	Geranyl diphosphate synthase
GPP	Gross primary productivity
LCU	Liquid calibration unit
m/z	Mass-to-charge ratio
MALDI	Matrix-assisted laser desorption/ionisation
MEP	Methylerythritol 4-phosphate
MFC	Mass flow controller
MPV	Multi-position valve
MSI	Mass spectrometry imaging

mTPS	Monoterpene synthase
MVA	Mevalonate
NanoSIMS	Nanoscale secondary ion mass spectrometry
NIST	National Institute of Standards and Technology
NMR	Nuclear magnetic resonance spectroscopy
PDH	Pyruvate dehydrogenase
PTR-TOF-MS	Proton transfer reaction time-of-flight mass spectrometer
RhNudx	Nudix hydrolase
ROS	Reactive oxygen species
RT	Retention time
SqTPS	Sesquiterpene synthase
TCA	Tricarbocylic acid
TDU-CIS	Thermodesorption-cold injection system
VPD	Vapour pressure deficit
VWC	Volumetric water content
ZAG	Zero-air generator

1. Introduction

1.1. Volatile organic compounds in a changing climate

Non-methane volatile organic compounds (VOCs) have profound impacts on atmospheric chemistry (Atkinson & Arey, 2003; Claeys *et al.*, 2004; Kanakidou *et al.*, 2005; Lelieveld *et al.*, 2008; Nölscher *et al.*, 2013; Bourtsoukidis *et al.*, 2014; Riccobono *et al.*, 2014; Unger, 2014), enhancing radiative forcing, significantly contributing to global warming (Masson-Delmotte *et al.*, 2021) and subsequent effects on human health (Chen *et al.*, 2011b; Tuet *et al.*, 2017) (**Figure 1**). About 75-90% of global VOC emissions can be assigned to natural ecosystems and these emissions are predominantly driven by vegetation (Guenther *et al.*, 1995; Lamarque *et al.*, 2010). These biogenic VOC (BVOC) emissions mainly compose of isoprenoids, contributing 68-83% (Isoprene 50-70%, monoterpenes 11-15%, sesquiterpenes 2.5-3%) to global BVOC emissions (Guenther *et al.*, 2012; Sindelarova *et al.*, 2014). Isoprenoids, specifically isoprene (C_5H_6), monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$), influence the photochemical ozone production (Atkinson & Arey, 2003; Bourtsoukidis *et al.*, 2014) and promote secondary organic aerosol (Riccobono *et al.*, 2014) and cloud formation (Unger, 2014). Even though global modelling approaches have compellingly improved throughout the last decades (Guenther *et al.*, 1991; Guenther *et al.*, 1993; Guenther *et al.*, 1995; Lathière *et al.*, 2006; Guenther *et al.*, 2012; Sindelarova *et al.*, 2014; Messina *et al.*, 2016), predictions of Isoprenoid and other BVOC emission development in response to a changing climate still bear high levels of uncertainty (Masson-Delmotte *et al.*, 2021; Thornhill *et al.*, 2021). How global emissions of isoprenoids and other BVOCs will alter under ongoing climate change is a matter of current debate, ranging from the prediction of dramatic increases of 30-45% with rising global temperatures (Peñuelas & Llusià, 2003), to stagnation or even decline due to predicted anthropogenic land-use change scenarios (Hantson *et al.*, 2017; Sindelarova *et al.*, 2022). Model parameterisation is limited by our understanding of fundamental processes driving isoprenoid emissions, their physicochemical controls and plant metabolic adjustments in dependence on environmental factors (Niinemets *et al.*, 2004; Messina *et al.*, 2016).

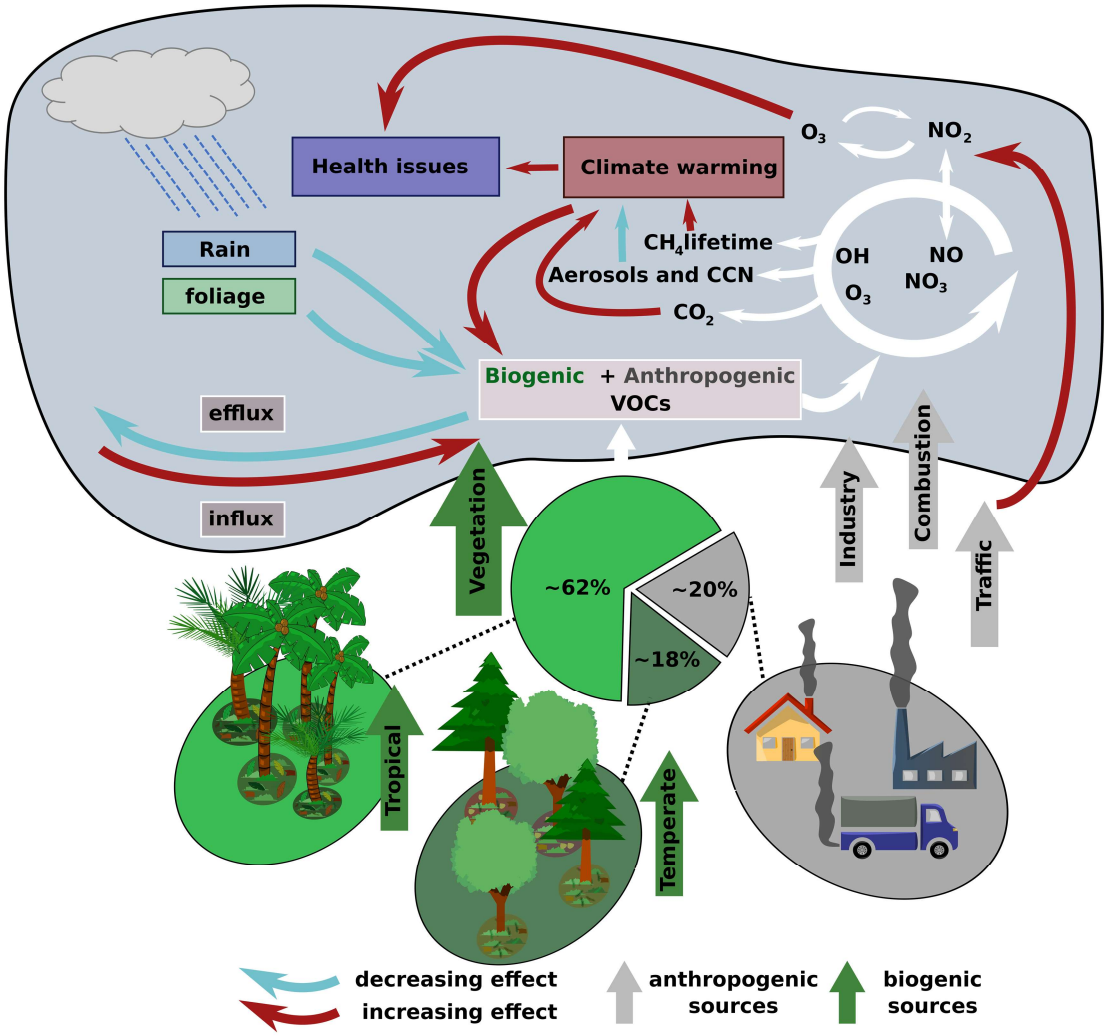

Figure 1 Schematic representation of atmospheric processes affected by volatile organic compounds from biogenic and anthropogenic sources. Especially metropolitan areas are exposed to interacting emissions of BVOCs, AVOCs, and other anthropogenic pollutants, with detrimental effects on air quality and human health. Presented estimates for global contributions are based on model comparisons between MEGANv2.1 (Guenther *et al.*, 2012) and ORCHIDEE (Lathière *et al.*, 2006; Messina *et al.*, 2016) and further studies (Guenther *et al.*, 1995; Lamarque *et al.*, 2010). Estimates for temperate forests include a contribution of < 5% from boreal forests. Modified after Peñuelas & Staudt (2010). Visualisation: Lars Erik Daber.

1.2. Functions, emission and biosynthesis of isoprenoids

Isoprenoids protect plants against herbivores, pathogens, reactive oxygen species and heat shock

Plants release a plethora of BVOCs that are essential for plant functioning and plant-environmental interactions (Loreto & Schnitzler, 2010). Floral scents serve to attract

pollinators (Raguso, 2008), above- and below-ground emissions from vegetative tissue deter herbivores (Unsicker *et al.*, 2009; Hiltpold & Turlings, 2012) and pathogens (Huang *et al.*, 2012) and attract beneficial organisms (Schulz-Bohm *et al.*, 2018). Among other BVOCs, such as stored green leaf volatiles, isoprenoids are released upon herbivore feeding, attracting herbivore enemies (Turlings *et al.*, 1990; Trapp & Croteau, 2001; Mäntylä *et al.*, 2008; Piva *et al.*, 2019) and activating defences in neighbouring plants (Baldwin *et al.*, 2006; Riedlmeier *et al.*, 2017). Isoprenoids protect plants against abiotic stressors, such as drought, extreme light and heat (Loreto *et al.*, 1998b; Loreto *et al.*, 2001; Dudareva *et al.*, 2006). Isoprene protects foliage against heat flecks, i.e. the large and rapid changes in leaf temperature caused by sunlight (Sharkey & Yeh, 2001; Velikova & Loreto, 2005; Behnke *et al.*, 2007), stabilising photosynthesis under extreme temperature spikes (Singsaas *et al.*, 1997). Further, isoprene can prevent losses in photosynthetic capacity by reactive oxygen species (ROS) (Peñuelas & Llusià, 2002; Sharkey *et al.*, 2008). However, the underlying mechanism responsible for the protective functions of isoprene remains unknown. Possibly, isoprene prevents heat induced phase transitions in phospholipid membranes of leaves, protecting plants against thermal shock by heat flecks (Siwko *et al.*, 2007; Sharkey *et al.*, 2008). Alternatively or additionally, isoprene might have direct ozone quenching properties (Loreto *et al.*, 2001), altering leaf internal ROS signalling (Vickers *et al.*, 2009). Similar mechanistic explanations revolve around the protective properties of monoterpenes (Peñuelas & Llusià, 2002, 2003; Loreto *et al.*, 2004; Copolovici *et al.*, 2005), and biosynthesis of isoprene and monoterpenes is tightly linked (Dudareva *et al.*, 2013).

Physiologic and physicochemical controls of isoprenoid emissions

Isoprenoid emissions of plants are affected by environmental, physiological and physicochemical factors. Isoprene emissions are light-dependent and diminish after a few seconds upon darkening, whereas monoterpene emissions can be supported for more than 15 minutes without *de novo* synthesis (i.e. synthesis from simple precursor molecules, such as sugars) by non-specific storage in the lipid phase of the leaf (Loreto *et al.*, 1998a; Shao *et al.*, 2001). In plants with specialised storage compartments, such as resin

ducts or glandular cells, monoterpenes can be emitted light independent for longer periods and even at night (Guenther *et al.*, 1993; Staudt *et al.*, 1997; Ghirardo *et al.*, 2010). Temperature regulates emission rates directly by affecting isoprenoid synthase activities (Monson *et al.*, 1992; Lehning *et al.*, 1999; Fischbach *et al.*, 2001) and indirectly by affecting respiration, changing isoprenoid precursor availability (Rosenstiel *et al.*, 2003). Further, temperature changes have a direct effect on monoterpene emissions from storage compartments and non-specific storage in the lipid phase of the leaf, since variations in temperature influence compound volatility (Fischbach *et al.*, 2002). Contribution of *de novo* synthesis to total monoterpene emissions in species with specialised storage compartments is species-specific and varies between 10 to 60% under unstressed conditions (Ghirardo *et al.*, 2010).

Under stressed conditions, for example under drought, emission development and contribution of *de novo* synthesis and storage pools is heterogenous. In the drought-resilient conifer *Pinus sylvestris*, *de novo* synthesis and emissions of monoterpenes were unaffected by a 10 day drought, but sesquiterpene emissions declined (Kreuzwieser *et al.*, 2021). Conversely, Wu *et al.* (2015) observed a slight increase in *de novo* synthesised monoterpene emissions of *Pinus sylvestris*, *Picea abies*, *Fagus sylvatica* and *Quercus ilex* during mild drought stress, but declining emissions with increasing drought severity. These trends have also been observed for ecosystem flux dynamics in response to drought (Grote *et al.*, 2009; Werner *et al.*, 2021; Byron *et al.*, 2022). Other studies focusing on branch measurements observed significant declines in monoterpene emissions in both, deciduous (Lavoir *et al.*, 2009) and conifer (Lüpke *et al.*, 2017) species. Hence, even though monoterpene emission dynamics under mild drought stress are highly diverse and likely ecosystem- and species-specific, *de novo* biosynthesis and emissions generally decline in response to long term drought stress, even in species with specialised storage compartments (Lewinsohn *et al.*, 1993; Trapp & Croteau, 2001). Notably, the temperature dependency of isoprene emission can be lost after severe drought (Fortunati *et al.*, 2008) and temperature dependency of monoterpene emission can be reduced by recurrent heatwaves (Birami *et al.*, 2021). However, it remains open how changes in *de novo* synthesis and storage pool size contribute to monoterpene emissions during drought.

Isoprenoid emissions of roots

Very little is known about magnitude, control and function of root emitted isoprenoids and other BVOCs, and even less is known about drought effects on below-ground emissions and storage. In addition to the ROS quenching properties, isoprene is hypothesised to be involved in root development (Miloradovic van Doorn *et al.*, 2020). Monoterpenes are likely involved in below-ground plant-environmental signalling (Rasmann *et al.*, 2005; Ali *et al.*, 2010) and might deter herbivores and pathogens. In conifer species isoprenoid emissions from roots are likely driven by storage pools (Lin *et al.*, 2007) but might be additionally emitted *de novo (Chen et al., 2004).* In response to drought, root isoprenoid emissions tend to decline (Lin *et al.*, 2007).

Initial discoveries and recent advances: Isoprenoids are produced via two (in-) dependent pathways

The two common precursors for biosynthesis of all isoprenoids are isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP) (**Figure 2**). For a long time it was assumed that these precursors are only synthesised in the cytosol via the mevalonate (MVA) pathway, starting from acetate that is activated as acetyl coenzyme A (Spurgeon & Porter, 1981; Rohmer *et al.*, 1993). It took more than half a century of contradicting results and discussions on the universal role of MVA as isoprenoid precursor, until Rohmer (1999) discovered an alternative, mevalonate-independent pathway for isoprenoid biosynthesis in plants: The plastidic methylerythritol 4-phosphate (MEP), also called non-mevalonate pathway converts glyceraldehyde 3-phosphate (GA-3-P) and pyruvate to IDP and DMADP. Though separated by compartments, these two pathways are connected by metabolic crosstalk of various precursors between the cytosol and plastid (**Figure 2**) (Laule *et al.*, 2003; Schuhr *et al.*, 2003; Bartram *et al.*, 2006; Jardine *et al.*, 2010; Ghirardo *et al.*, 2011; Opitz *et al.*, 2014; Sharkey & Monson, 2014; Rodríguez-Concepción & Boronat, 2015). However, relevance and magnitude of these crosstalk processes in plants remain controversial (McCaskill & Croteau, 1995; Dudareva *et al.*, 2005; de Souza *et al.*, 2018; Rasulov *et al.*, 2018), especially since the transport mechanisms still remain unclear (Bick & Lange, 2003; Dudareva *et al.*, 2006; Dudareva *et al.*, 2013).

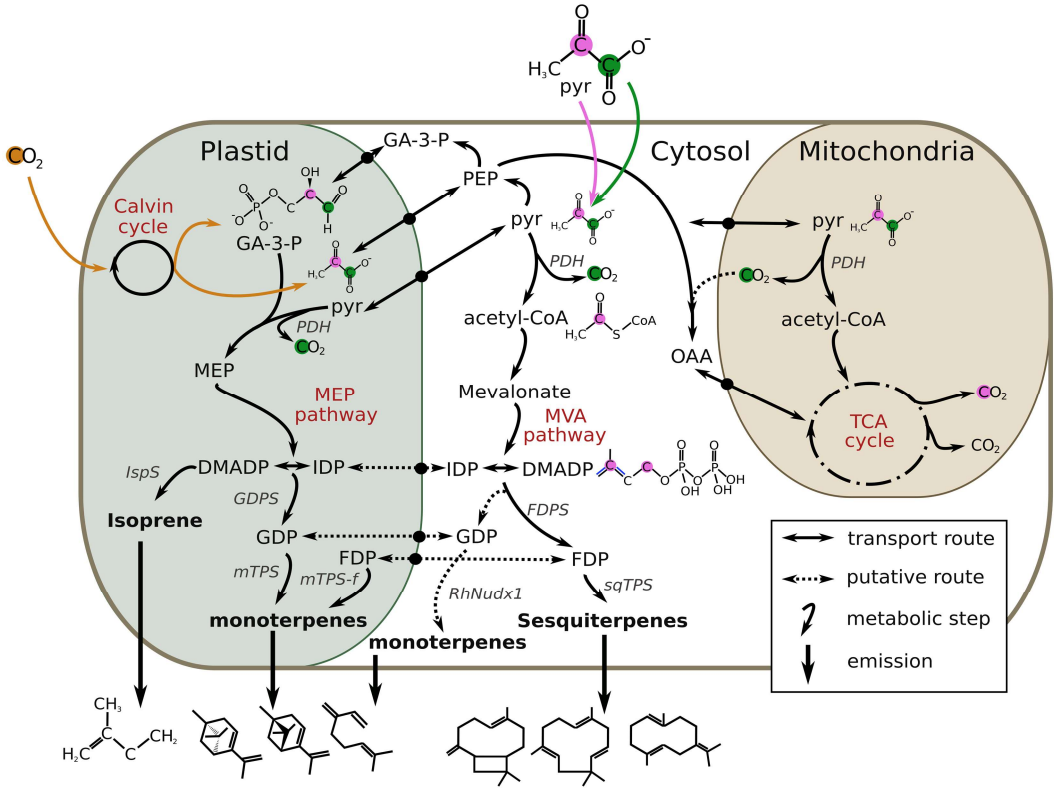

Figure 2 Metabolic pathways leading to the biosynthesis of isoprenoids in plant cells. Pathway names are highlighted in red, enzymes are in italic and volatile isoprenoids are in bold. The scheme was modified as synthesis from **Study 3** and **Study 4**. DMADP, dimethylallyl diphosphate; FDP, farnesyl diphosphate; FDPS, farnesyl diphosphate synthase; GA-3-P, glyceraldehyde-3-phosphate; GPP, geranyl diphosphate; GPPS, geranyl diphosphate synthase; IspS, isoprene synthase; IDP, isopentenyl diphosphate; IDPS, isopentenyl diphosphate synthase; MEP, methylerythritol phosphate; mTPS, monoterpene synthase; MVA, mevalonate; Pyr, pyruvate; PDH, pyruvate dehydrogenase; RhNudx, Nudix hydrolase; sqTPS, sesquiterpene synthase; TCA, tricarbocylic acid. Partially adapted from Dudareva *et al.* (2006), Tcherkez *et al.* (2017), Fasbender *et al.* (2018), Werner *et al.* (2020) and Zhou & Pichersky (2020a).

Isoprene biosynthesis is located in the plastids and promoted by conversion of IDP/DMADP via isoprene synthase (IspS, **Figure 2**) (Rohmer, 1999). Some IspS might be located in the cytosol, but their activity and contribution to overall biosynthesis is likely minor (Zhou & Pichersky, 2020b). IDP and DMADP are condensed to form geranyl diphosphate (GDP), the general precursor for monoterpene biosynthesis. There exists a multitude of monoterpene synthases (mTPS) in the plant kingdom that convert GDP to various monoterpenes and their diversity is further increased by subsequent modification through, e.g. oxidation, dehydrogenation and acylation (Trapp & Croteau, 2001;

Dudareva *et al.*, 2004). In general, biosynthesis of monoterpenes is also located within plant plastids (Cheniclet & Carde, 1985; Schürmann *et al.*, 1993). However, cytosolic monoterpene biosynthesis has been shown to occur in reproductive (Magnard *et al.*, 2015) and vegetative (Liu *et al.*, 2018; Zhou & Pichersky, 2020a) plant tissue in some cases, but the magnitude of cytosolic monoterpene biosynthesis compared to plastidic rates remains unresolved. Sesquiterpenes, on the other hand, are generally synthesised in the cytosol, where IDP and DMADP are condensed to form farnesyl diphosphate (FDP), which is then converted to a plethora of different sesquiterpenes (Dudareva *et al.*, 2006). FDP and GDP also resemble the building blocks for biosynthesis of homoterpenes and diterpenes, respectively.

1.3. Chiral monoterpenes

The TPS-enzyme family is characterised by some unusual abilities: Several multi-substrate TPS were identified in the TPS-f-subfamily, producing monoterpenes from both, GPP or FDP (Ruiz-Sola *et al.*, 2016; Dhandapani *et al.*, 2020). Even more substantial is the ability of most mTPS to produce multiple, partially chiral, monoterpenes at specific rates (Aubourg *et al.*, 2002; Chen *et al.*, 2011a; Wang *et al.*, 2016; Zhou & Pichersky, 2020b, 2020a). Chiral monoterpenes and other chiral compounds vary in the conformation of one or several asymmetric carbon atoms, reflecting non-superposable mirror images of each other (Bentley, 2006b). Chiral mixtures of monoterpenes can be produced by non-enantiospecific transformation by one enzyme, by enantiospecific transformation by two different enzymes in parallel, or by synthesis of a racemate, followed by further transformation steps that preferentially use one enantiomer (Norin, 1996). Chiral monoterpenes are identical in their physical properties, aggravating analytical distinction of enantiomers, since common ionisation techniques used in mass spectrometry (MS) have no chiral selectivity (Powis & Janssen, 2017). Additionally, atmospheric chemical reaction rates are not affected by chirality, which is why they are often overlooked in studies focusing on atmospheric monoterpene concentrations (Zannoni *et al.*, 2020; Byron *et al.*, 2022). However, their mirrored conformation significantly changes biochemical functioning by affecting e.g. antimicrobial/insecticidal properties and attractiveness to other organisms

(Phillips *et al.*, 1999; van Vuuren & Viljoen, 2007; Rufino *et al.*, 2014; de los Santos & Wolf, 2020). Since the first identification of enantiomer-specific taste of *D*-and *L*-asparagine by Piutti (1886), the chirality of natural compounds, their biosynthesis and biologic functioning have not only gained significant interest in the perfume, food- and pharmaceutical industry (Piutti, 1886; Bentley, 2006b), but also caught attention in the field of ecophysiology to study plant-insect interactions (Klimetzek *et al.*, 1980; Billings & Cameron, 1984; Persson *et al.*, 1993; Attygalle *et al.*, 2009).

Chiral ratios of above- and below-ground monoterpene emissions play essential roles in plant-insect communication (Lindström *et al.*, 1990; Norin, 1996; Erbilgin *et al.*, 2007; Ghimire *et al.*, 2016) and likely affect chemotaxis (Schulz-Bohm *et al.*, 2018). On the other hand, the insecticidal and antimicrobial properties of several chiral compounds produced by e.g. *Citrus* sp., *Mentha* sp., or coniferous tree species are seen to deter herbivores (Borg-Karlson *et al.*, 1993; Lücker *et al.*, 2002) and pathogens (Mikaili *et al.*, 2013). (-)- and (+)-enantiomers of limonene obtain highly antimicrobial properties (Aggarwal *et al.*, 2002), whereas only the (+)-enantiomers of α- and β-pinene were found to possess antimicrobial functions (Da Rivas Silva *et al.*, 2012). Further, biological activity and insecticidal/antimicrobial characteristics can be modified depending on chiral ratios of monoterpenes (van Vuuren & Viljoen, 2007).For other compounds, such as β-phellandrene and camphene, there is no information on insecticidal/antimicrobial activity of isolated enantiomers available, yet. Nonetheless, shifts in chiral monoterpene composition observed after application of external stressors, such as mechanical wounding (Yassaa & Williams, 2007; Williams *et al.*, 2011; Bougas *et al.*, 2022) or drought stress (Byron *et al.*, 2022) likely affect interactions of plants with their environment. However, changes in emission and biosynthesis of chiral ratios as a consequence of drought are vastly understudied on a plant scale.

1.4. Scope of this thesis

Isoprenoids shape the chemical landscape of many ecosystems, protecting plants from external stressors and mediating plant-environmental signalling. In addition, isoprenoids have profound effects on atmospheric chemistry and climate warming. Our understanding of emission regulation and biosynthesis of isoprenoids from various plant species of different ecosystems has significantly improved throughout the last decades (Norin, 1996; Niinemets *et al.*, 2004; Dudareva *et al.*, 2006; Loreto & Schnitzler, 2010; Peñuelas & Staudt, 2010; Zhou & Pichersky, 2020a). However, in regard to climate change, severe droughts will globally increase (Allen *et al.*, 2015) with unforeseen ramifications for ecosystem functioning (Grossiord *et al.*, 2014), plant-environmental interactions (Anderegg *et al.*, 2015) and atmospheric isoprenoid concentrations (Guenther *et al.*, 2012; Messina *et al.*, 2016). Understanding the fundamental processes driving plant biosynthesis and emission of isoprenoids in different ecosystems and environmental conditions is therefore essential for many scientific fields: Model parameterisation requires information on emission changes under a changing climate with increasing drought severity and frequency; changes in volatile plant signals are cues for understanding plant-environmental interactions and how they are affected by drought; and possible shifts in chiral mixtures under changing environmental conditions can imply vital information for advances in drug development. Drought affects how fresh photosynthates and alternative carbon sources are utilised for isoprenoid biosynthesis (Brilli *et al.*, 2007) but the significance of metabolic crosstalk between plant cell compartments remains highly debated (Rasulov *et al.*, 2018). We especially lack information on how emission and biosynthesis of chiral compounds is regulated during drought. Further, relations between storage derived emissions and storage pool size as well as composition in response to drought have not been assessed. Lastly, drought effects on below-ground dynamics of isoprenoids and specifically chiral monoterpenes, remain unexcavated until now. Therefore:

It is the scope of this thesis, to advance our understanding on the effects of drought on above- and below-ground emission and biosynthesis of isoprenoids. A specific focus lies on investigating these dynamics for chiral monoterpenes in response to drought.

1.5. Experimental design and approaches

Biosynthesis and emission of isoprenoids are affected by plant physiological status, which depends on the highly diverse current and past environmental conditions of their habitat (Peñuelas & Llusià, 2003; Niinemets *et al.*, 2004). To understand how specific environmental stressors, such as drought, affect plant biosynthesis and emission of isoprenoids, confounding factors can be exterminated by conducting experiments on potted plants under standardised conditions in climate chambers. Heavy, complex and energy demanding instrumentation can be installed, simplifying experimental efforts and enhancing data resolution compared to field studies. Even though conditions in potted plants are far from natural conditions, these experiments are crucial to understand underlying mechanisms that drive emission dynamics in the natural world (Diamond, 1986). Nonetheless, metabolic functioning might be significantly affected by plant developmental status and age (Borg-Karlson *et al.*, 1993; Laothawornkitkul *et al.*, 2009). Fortunately, a unique research facility has been built in the Arizonan desert to fill the gap between field- and laboratory studies.

The Biosphere 2 Water and Life Dynamics drought experiment

Though originally built in the 90's in Tucson, Arizona (USA) to study the suitability of space colonisation by creating an independent, second biosphere (Cohen & Tilman, 1996; Allen & Nelson, 1999), Biosphere 2 (B2) has been developed to an exceptional research platform for large-scale environmental experiments throughout the last decades (Lin *et al.*, 1998; Hopp *et al.*, 2009; Barron-Gafford *et al.*, 2019; Roach *et al.*, 2020). The tropical rainforest mesocosm at Biosphere 2 is an enclosed ecosystem that enables comprehensive studies about environmental effects on individual ecosystem components (Ananyev *et al.*, 2005; van Haren *et al.*, 2005; Pegoraro *et al.*, 2006; Taylor *et al.*, 2018; Barba *et al.*, 2019; Smith *et al.*, 2020; Werner *et al.*, 2021). The tropical rainforest contains a diverse mixture of pantropical shrub and tree species, many of which have been planted 30 years ago. Humidity, temperature and irrigation can be fully controlled and measured on all levels of the ecosystem.

With this setup, a 9.5-week drought was imposed on the system during the Biosphere 2 Water and Life Dynamics (B2WALD) campaign. As part of the B2WALD-team, I was involved in infrastructural installations, as well as the development, planning, execution and analysis of experiments throughout the 6 months of the campaign (**Study 1-3**) (Honeker *et al.*, 2022). Drought influenced ecosystem carbon and water fluxes, significantly declining evapotranspiration, ecosystem respiration and gross primary productivity (GPP), but enhancing BVOC emissions (**Study 1**).

Within the scope of the overarching drought experiment, representative plant species of the ecosystem were selected and classified in four plant functional groups (drought-sensitive/-tolerant understory, and canopy forming trees) for further investigations. Selection was based on canopy coverage, distribution within the ecosystem, and number of available replicates (for details, see **Study 1**). For **Study 3**, two of the representative canopy-forming tree species (*Clitoria fiarchildiana* R. A. Howard, drought-sensitive; *Phytolacca dioica* L., drought-tolerant) and two representative understory species (*Hisbiscus rosa sinensis* L., drought-tolerant; *Piper auritum* Kunth, drought-sensitive) were selected for experiments (**Study 1**). In case of **Study 2**, experiments were performed on *C. fairchildiana*, *H. rosa sinensis*, and *P. auritum*. Experiments were conducted before and during the B2WALD drought. To measure fluxes of BVOCs, H_2O and CO_2 on-line throughout the whole ecosystem, a sophisticated pneumatic multi-positions leaf cuvette measurement system was setup inside the rainforest (for details, see **Study 1 and 3**).

Automated, pneumatic multi-position leaf cuvette gas sampling systems

Several experimental techniques can be exploited to sample volatile organic emissions of plants, ranging from simplistic bag sampling approaches (Capelli *et al.*, 2013) to sophisticated, automated multi-cuvette sampling systems (Werner *et al.*, 2021). Since BVOCs are generally emitted in low quantities (ppt-ppm), distinction between plant emissions and surrounding (i.e. background) concentrations is challenging. One possibility to handle high background concentrations is to accumulate emissions, e.g. by closing sampling containers around the plant material prior to sampling (Tholl *et al.*, 2006; Meischner *et al.*, 2022), closed-loop stripping systems (Donath & Boland, 1995) , or by pre-

concentration on chemical filters (Linskens & Jackson, 1997; Saebo *et al.*, 2013; Kreuzwieser *et al.*, 2021). Alternatively, background concentrations can be eliminated by filtering background air through activated charcoal and/or platinum catalysators (Dudareva *et al.*, 2003; Kunert *et al.*, 2005; Fasbender *et al.*, 2018) or by flushing cuvettes with synthetic air (Ghirardo *et al.*, 2010). Each of these approaches comes with different advantages and issues, which should be carefully assessed in dependence of the research question (Tholl *et al.*, 2006; Kumar & Víden, 2007). BVOC emissions are strongly affected by short-term changes of temperature, light and physical contact (Niinemets *et al.*, 2004). Hence, to measure plausible BVOC fluxes and their response to isolated stressors, plants should be equilibrated in a stable environment for several hours before sampling. To continuously measure BVOC fluxes of several plants in equilibrated conditions over longer time periods in parallel, automated multi-cuvette sampling systems are currently utilised as state-of-the-art (**Study 5**). As part of the B2WALD-team and of this thesis (**Study 1 and 3**), an automated multi-cuvette sampling system was developed and constructed within the tropical rainforest biome at B2. For **Study 4 and 5** an existing automated multi-cuvette sampling system inside of two walk-in climate chambers basing on the design from Fasbender *et al.* (2018) was rebuilt and improved. Even though complexity between the systems differed, the general functioning principle was similar (for details see **Study 1** and **Study 4**).

Identification and determination of BVOC concentrations

Proton transfer reaction time-of-flight mass spectrometry is currently the prevalent tool to identify and quantify the whole spectrum of VOCs in gas samples on-line (Jordan *et al.*, 2009). Pulsed, charged ion beams are focused and accelerated towards a detector, usually a microchannel plate coupled to an analogue-to-digital converter. To charge the volatile analytes, proton transfer is mediated by chemical ionisation (CI), generally with H_3O^+ ions. CI is a soft ionisation method that is characterised by low fragmentation rates. CI significantly simplifies subsequent data analysis since mass-to-charge ratios (m/z) of analytes are only changed by the addition of one H^+ ion (**Equation 1**).

$$VOC + [H_3O^+] \rightarrow [VOC + H^+] + H_2O, \quad 1)$$

Separation of ions of different m/z is achieved by acceleration through a field-free drift path of known length (Wolff & Stephens, 1953). Mass resolution power is determined by path length, which has been significantly improved from linear- (Wolff & Stephens, 1953) to multi-reflecting TOF-MS (Satoh *et al.*, 2014) throughout the last century. Mass determination is achieved by referencing TOF to compounds of known weight throughout the spectrum, called mass-axis calibration. Reliable quantification of analytes requires regular calibration with gas mixtures of known compounds and concentration to relate the detector signal (i.e. counts per second) to an actual concentration of the respective analyte (Holzinger, 2015; Holzinger *et al.*, 2019).

PTR-TOF-MS resolves and quantifies volatile analytes by mass. The wide range of compounds that can be covered with just one instrument is intriguing. However, PTR-TOF-MS lacks to resolve structural differences among compounds. Hence, other MS techniques, such as gas chromatography electron ionisation (GC-EI) MS is required to resolve and quantify e.g. different monoterpenes separately (Lindeman & Annis, 1960). GCs can separate volatile analytes in complex mixtures via capillary separation columns (Kuck, 2002). For analysis of VOCs, volatiles can be sampled on glass tubes filled with Tenax (Sigma, Munich, Germany) which are then inserted into the GC-MS. Volatiles are trapped on a thermodesorption-cold injection system (TDU-CIS, Gerstel, Germany). A temperature gradient is applied from low to high temperatures, while the sample is flushed with Helium as a carrier gas. Depending on chemical binding strength between the analyte and binding chemical, the analytes are released and carried towards the EI through a GC separation column at a specific temperature and retention time (RT). Separation of chiral monoterpenes can be achieved by installation of an appropriate column (e.g. beta-Dex 120 Chirality, 60 m × 250 µm × 0.25 µm, Supelco, USA). Fragmentation patterns of the analyte created by EI are compound specific and can be exploited for identification via extensive compound libraries, such as NIST library (National Institute of Standards and Technology). Identification can be verified by analysing and comparing RTs and fragmentation pattern of commercially available standards with different samples (**Study 5**). By

splitting the eluate towards an isotope ratio (IR) MS, ^{13}C isotope ratios of according volatiles can be additionally determined.

Position-specific ^{13}C pyruvate labelling

Isotopic tracer experiments are an effective tool to study biosynthetic regulation of isoprenoids. The utilisation of fresh photosynthates for biosynthesis of plant metabolites can be analysed *in vivo* by manipulating the isotopic ratio of carbon in CO_2 that is supplied to branches installed in leaf cuvettes (**Figure 2**). By supplying plants with 100% $^{13}CO_2$ instead of ambient concentrations (1.1%), it is possible to estimate the contribution of *de novo* synthesis to overall isoprenoid emissions (Ghirardo *et al.*, 2010). The contribution of cytosolic carbon sources to isoprenoid biosynthesis, on the other hand, can be investigated by supplying branches with isotopically labelled, central metabolites, such as ^{13}C-glucose (Ghirardo *et al.*, 2011), or ^{13}C-pyruvate (Jardine *et al.*, 2014), via the transpiration stream (**Figure 2**). The application of position-specifically labelled pyruvate allows to further understand which metabolic processes lead to the biosynthesis of different plant metabolites (Fasbender *et al.*, 2018; Werner *et al.*, 2020; Ladd *et al.*, 2021). These presented methods are by now well-established and widely applied to study BVOC emissions from leaves of various plant genera under diverse environmental conditions (Jardine *et al.*, 2010; Yáñez-Serrano *et al.*, 2019; Kreuzwieser *et al.*, 2021). However, to my knowledge position-specific pyruvate labelling has not been employed to study these dynamics in roots.

These methodologic approaches were selected to appropriately address the scope of this thesis. Thus, I conducted position-specific labelling experiments on leaves and roots of tropical and boreal plant species and measured plant physiologic responses to drought and labelling with an automated multi-cuvette sampling system, including PTR-TOF-MS and sampling for GC-MS-IRMS analysis of isoprenoids. In the following, the detailed research objectives and hypotheses are presented, specifying current knowledge gaps within the scope of this thesis.

1.6. Research objectives and hypotheses

In accordance with the scope of this thesis, the overarching aim of the conducted studies was to investigate whether and how above- and below-ground emission and biosynthesis of various, partially chiral, isoprenoids are affected by drought in tropical and boreal plant species.

As part of this thesis and of the B2WALD-team, I contributed to the development and set up of the experimental measurement system within the B2 tropical rainforest, including an automated multi-cuvette sampling system, several kilometres of PFA tubing for gas sampling of various plant species, and diverse sensors (see **chapter 5** for specific contributions). Within the framework of the overarching B2WALD drought experiment (**Study 1**), I performed position-specific stable isotope experiments to investigate changes in carbon utilisation for plant respiratory processes and isoprene and monoterpene biosynthesis before and during drought (**Study 3**) and developed a method to isotopically label roots for comparative analysis (**Study 2**). After the B2WALD campaign, I built an automated multi-cuvette sampling system within two walk-in climate chambers based on the initial set up described by Fasbender *et al.* (2018). I conducted a drought experiment on potted Norway spruce saplings to assess differences in chiral monoterpene biosynthesis above- and below-ground by applying several isotopic tracers (**Study 4 and 5**). The research objectives and hypotheses are presented in the following:

(1) The first objective is to investigate how drought mediated changes in ecosystem fluxes are reflected among metabolic processes and isoprenoid emission of different plant functional groups.

The increasing severity and frequency of droughts in current and future climate scenarios (Spinoni *et al.*, 2020) could result in increasing isoprenoid emissions from tropical forests (Loreto & Schnitzler, 2010; Guenther *et al.*, 2012) and puts their functioning as major carbon sinks at risk (Pan *et al.*, 2011; Wigneron *et al.*, 2020). Yet, our understanding of species-specific regulation of carbon investment into maintenance and protection by drought-stressed plants above- and below-ground, by e.g. maintaining BVOC emissions, is insufficient (Holopainen & Gershenzon, 2010; Loreto & Schnitzler, 2010; Hartmann *et al.*, 2020). To understand how drought affects ecosystem fluxes, the underlying processes

driving these dynamics need to be evaluated. Thus, in **Study 1-3**, I assess the effect of an unprecedented drought in the B2 tropical rainforest on above- and below-ground carbon utilisation for isoprenoid biosynthesis of representative canopy and understory species of different plant functional groups, using position-specific stable isotope labelling. Originally, the aim was to additionally compare the drought response of above- and below-ground BVOC emissions of representative plant species. Unfortunately, we faced substantial technical problems with the PTR-MSs measuring root emissions during the campaign, which is why I evaluate the interplay of above-ground emission dynamics with below-ground metabolic processes instead:

I hypothesise, that adjustments in water and carbon fluxes and carbon utilisation for different metabolic processes in response to drought are species-specific (Study 1-3).

After the assessment of drought mediated metabolic responses from tropical plant species, I focus on investigating how drought affects the emission and biosynthesis of chiral monoterpenes in needles and roots of *Picea abies* in **Study 4** and **Study 5**:

(2) The second and main objective is to assess how drought affects above- and below-ground emission and biosynthesis of chiral monoterpenes from *Picea abies*, by:

(i) **Introducing different isotopic tracers into needles and roots of *Picea abies* to investigate biosynthetic patterns among chiral monoterpene emissions; and**

(ii) **comparing the composition and concentration of chiral monoterpenes emitted and stored by needles and roots of *Picea abies* in response to drought.**

The assessment of drought effects on the biosynthesis of the wide range of chiral monoterpenes stored and emitted by conifers (Persson *et al.*, 1993; Sjödin *et al.*, 1996) is aggravated by the challenging analytical distinction (Powis & Janssen, 2017). Earlier studies suggest that monoterpene composition differs between above- and below-ground tissue (Borg-Karlson *et al.*, 1993; Sjödin *et al.*, 1996), which could indicate shoot-independent biosynthesis in roots. I therefore introduce stable isotope tracers into needles and roots of *Picea abies* to investigate how chiral monoterpenes are synthesised in a species that emits chiral monoterpenes *de novo* and from storage pools in **Study 4** and **Study 5**:

I hypothesise, that chiral monoterpenes are partially synthesised *de novo* in needles and roots of *Picea abies*, with *de novo* synthesis diminishing as a consequence of drought stress (Study 4 and Study 5).

Biological activity and biochemical functioning differs between different monoterpenes and their enantiomers (Borg-Karlson *et al.*, 1993; Aggarwal *et al.*, 2002; Lücker *et al.*, 2002; van Vuuren & Viljoen, 2007; Da Rivas Silva *et al.*, 2012; Mikaili *et al.*, 2013) and it is likely that chiral monoterpenes serve different functions in plant-atmospheric and plant-soil interactions (Lindström *et al.*, 1990; Norin, 1996; Erbilgin *et al.*, 2007; Ghimire *et al.*, 2016; Schulz-Bohm *et al.*, 2018). Even though external stressors can lead to shifts in chiral monoterpene composition and ratios in plant emissions (Yassaa & Williams, 2007; Byron *et al.*, 2022), drought-related adjustments in composition of chiral monoterpene emissions and storage pools, especially in below-ground tissue, have received little attention until now. Thus, in **Study 4** and **Study 5**, I analyse variations in chiral monoterpene composition of emissions and storage pools from *Picea abies* needles and roots in response to drought:

I hypothesise, that composition and concentration of chiral monoterpenes differ above- and below-ground and are affected by drought; and that compositional differences constitute cues on chiral monoterpene functioning (Study 4 and Study 5).

The light-independent portions of monoterpene emissions from species with specialised storage compartments are generally seen to be passively emitted from these compartments in dependence to temperature (Fischbach *et al.*, 2002). Therefore, passive emissions from storage compartments should only be limited by storage pool size and temperature. Further, composition of emissions from storage pools should match the composition of stored monoterpenes. In **Study 5**, I tested these relations for needles and roots of Norway spruce by analysing chiral monoterpene concentration and composition of emissions and storage pools under diminishing contributions from *de novo* synthesis:

I hypothesise, that the composition and concentration of chiral monoterpenes emitted from needles and roots of *Picea abies* from storage pools are tightly linked to storage pool size and composition (Study 5).

Overall, this study addresses these research objectives and hypotheses by combining position-specific stable isotope labelling techniques with state-of-the-art measurements of isoprene and chiral monoterpenes via PTR-TOF MS and GC-MS-IRMS, possibly stimulating further investigations at the frontier of our current understanding of plant isoprenoid biosynthesis and emission regulation.

2. Main results and discussion

2.1. The Biosphere 2 Water and Life Dynamics (B2WALD) drought experiment

*In **Study 1-3**, which were conducted within the overarching framework of the large-scale drought experiment in an experimental tropical rainforest (B2WALD campaign), I investigated how drought affects metabolic carbon utilisation by different plant functional groups in relation to overall ecosystem fluxes, with a specific focus on isoprenoid biosynthesis.*

The enclosed tropical rainforest mesocosm at B2 constitutes a unique research facility for large-scale ecosystem manipulation experiments, since environmental parameters such as humidity, irrigation and temperature can be fully controlled. To determine the underlying mechanisms driving drought mediated changes in overall ecosystem water and carbon dynamics, the tropical rainforest was exposed to a 9.5-week drought in 2019 (**Study 1**).

2.1.1. Ecosystem fluxes are disproportionally affected by different plant functional groups

Drought led to dramatic declines in ecosystem H_2O and CO_2 fluxes, reducing GPP by 79% during severe drought (**Study 1**). Daytime atmospheric isoprene concentrations increased already in early drought but subsequently diminished during severe drought, when monoterpene concentrations peaked (**Study 1**). At the end of severe drought, monoterpene and hexane concentrations increased before declining to pre-drought levels when the ecosystem was released from the drought stress during the recovery period. These dynamics in ecosystem fluxes were affected by four distinct plant functional groups: drought-sensitive/drought-tolerant canopy trees and drought-sensitive/drought-tolerant understory species (**Study 1**). Ecosystem water fluxes in response to drought were driven by drought-sensitive canopy trees. However, the contribution of drought-

tolerant canopy trees and understory increased during drought when fluxes from drought-sensitive canopy trees declined (**Study 1**).

To evaluate the underlying metabolic adjustments of these four plant functional groups to the drought stress, isotope labelling experiments were performed on leaves and roots of representative species from each group (**Study 2** and **Study 3**). Leaf and root labelling experiments with position-specifically labelled ^{13}C1-/^{13}C2-pyruvate were performed during the pre-drought (12-28 September 2019) and the beginning of the severe drought (4-16 November 2019) of the B2WALD campaign (**Study 1**). After root labelling, roots were prepared and split for subsequent imaging via matrix-assisted laser desorption/ionisation coupled to mass spectrometry imaging (MALDI-MSI), nanoscale-secondary ion mass spectrometry (NanoSIMS), and metabolite extraction via water extraction for ^{1}H NMR bulk metabolite characterisation (**Study 2**).

Carbon and water fluxes of the drought-tolerant understory species *Hibiscus rosa sinensis* were two to four times lower than in the drought-sensitive canopy tree *Clitoria fairchildiana* during pre-drought conditions and were unaffected by drought (**Study 3**). However, root metabolic profiles and carbon utilisation were altered by drought in *H. rosa sinensis*: Concentrations of fatty acids and antioxidants were upregulated below-ground (**Study 2**), and increased release of $^{13}CO_2$ from ^{13}C2-pyruvate by leaves indicated elevated daytime TCA cycle activity above-ground (**Study 3**). These processes likely supported drought resilience under elevated ROS levels in *H. rosa sinensis* (Lawlor & Khanna-Chopra, 1984; Hoefnagel *et al.*, 1998; Noctor *et al.*, 2002; Bartoli *et al.*, 2004; Atkin & Macherel, 2009; Zia *et al.*, 2021). Similar trends were observed for the drought-tolerant canopy tree *Phytolacca dioica* (**Study 3**).The non-woody, drought-sensitive understory species *Piper auritum* reinforced cell walls in response to drought to increase vasculature mechanistic ability and to reduce water leakage (Cabane *et al.*, 2012) (**Study 2**). Nonetheless, carbon and water fluxes significantly declined and leaf senescence increased during drought in *P. auritum* (**Study 1** and **Study 3**). Leaf senescence and leaf shedding was even higher in drought-sensitive canopy species and was particularly pronounced in the high isoprene emitting legume tree *C. fairchildiana*, contributing to declining atmospheric isoprene concentrations after early drought (**Study 1**). Additionally, isoprene and

monoterpene emissions from remaining leaves were downregulated at the beginning of severe drought (**Study 3**). Despite the drought-mediated declines in assimilation, transpiration and isoprenoid emissions in *C. fairchildiana*, drought had little effect on root metabolic profiles and above-ground carbon utilisation for anabolic and catabolic processes (**Study 2** and **Study 3**). Rather, root metabolic profiles of *C. fairchildiana* were affected by presence/absence of symbiotic microbial interactions at root nodules (**Study 2**). Leaf senescence and shedding in response to drought is a common strategy by plants to ensure plant survival under severe drought stress to reduce water loss and relocate nutrients (Munné-Bosch & Alegre, 2004), which might have supported maintenance of below-ground metabolism in *C. fairchildiana*.

Thus, the combination of position-specific isotope labelling with different MS techniques elucidated plant-specific responses and above- and below-ground metabolic mediation strategies in response to drought. These findings demonstrate the importance to evaluate both, above- and below-ground adjustments to drought, to fully understand drought response strategies of different plant species and their impact on overall ecosystem processes, such as carbon and water fluxes.

2.1.2. Differences in label incorporation into isoprene and monoterpenes in a tropical legume tree in response to drought

Root metabolic profiles of *C. fairchildiana* were determined by the symbiotic interaction with nitrogen-fixing microbes (**Study 2**). These below-ground interactions might explain the remarkable difference in above-ground ^{13}C-pyruvate daytime utilisation for different metabolic processes even under pre-drought conditions (i.e. the low decarboxylation rate of the C1-position of pyruvate), compared to the other three species (**Study 3**). Cytosolic pyruvate might be utilised for anaplerotic reactions without decarboxylation of the C1-position of pyruvate, supplying amino acid synthesis (Hanning & Heldt, 1993; Tcherkez *et al.*, 2009; Sweetlove *et al.*, 2010), due to the higher nitrogen availability in legume trees. *C. fairchildiana* was the only species allocating significant amounts of ^{13}C label to isoprenoids and ^{13}C incorporation into isoprenoids was similar for ^{13}C1- and ^{13}C2-pyruvate (**Study 3**). Hence, alternatively or additionally to anaplaerotic

processes, pyruvate was likely utilised for biosynthesis of isoprenoids via GA-3-P, which conserves all three carbon atoms from pyruvate, instead of decarboxylating the C1-position (**Figure 2, Study 3**) (Werner *et al.*, 2020; Ladd *et al.*, 2021). Interestingly, earlier studies have suggested a tight link between leaf nitrogen fixation and isoprene synthesis (Rosenstiel *et al.*, 2003; Rosenstiel *et al.*, 2004).

Label incorporation of cytosolic ^{13}C-pyruvate into isoprene increased as the supply of fresh photosynthate available for isoprenoid biosynthesis declined in response to drought (**Study 3**). Similar patterns have been observed for heat-stressed plants in growth chambers (Yáñez-Serrano *et al.*, 2019) or when assimilation was artificially reduced due to abscisic acid mediated stomatal closure (Schnitzler *et al.*, 2004). Surprisingly, label incorporation into monoterpenes did not increase in parallel during drought (**Study 3).** If isoprenoid biosynthesis of both isoprene and monoterpenes was supplied by the same metabolic pathways, label incorporation should have been similar. The observed differences in label incorporation indicate that cytosolic pyruvate supplies isoprenoid biosynthesis through (at least) two different pathways. Isoprene and monoterpenes are both supplied via GA-3-P, but additionally, monoterpene biosynthesis might be supplied by cytosolic metabolites that are not available for biosynthesis of isoprene (for a detailed discussion, see **Study 3**).

The results of these studies demonstrated that plant species of different plant functional groups vary in their metabolic responses to drought and might disproportionally contribute to overall ecosystem fluxes. Diminishing atmospheric isoprene concentrations in response to drought were partially driven by the legume tree *C. fairchildiana*, which increasingly resorted to cytosolic pyruvate for isoprene emissions, as the supply of fresh photosynthate available for biosynthesis declined (Study 1-3).

2.2. Position-specific pyruvate label indicates significant *de novo* synthesis of chiral monoterpenes in needles but not in roots of Norway spruce

*Following the B2 drought experiment, I investigated dynamics in isoprenoid emission and biosynthesis in the native conifer tree Picea abies in **Study 4** and **Study 5**. As part of these studies, I analysed the contribution of de novo synthesis to overall emissions and storage pools of chiral monoterpenes in needles and roots of Picea abies.*

Three different isotopic tracers were introduced into needles of Norway spruce to evaluate the contribution of *de novo* synthesis to overall chiral monoterpene emissions: (*i*) $^{13}CO_2$ via fumigation of branches (**Figure 2**), (*ii*) ^{13}C1-pyruvate and (*iii*) ^{13}C2-pyruvate dissolved in H_2O by cutting and placing branches inside the isotopic solution (**Study 4**). For investigating whether chiral monoterpenes are synthesised *de novo* in roots of Norway spruce, the tips of roots were placed in ^{13}C2-pyruvate solution (**Study 5**). As expected, significant amounts of label were integrated into needle emissions (**Study 4**). In contrast, *de novo* synthesis was not observed in root emitted monoterpenes, even though other BVOCs, such as acetaldehyde, ethanol and acetone were significantly labelled (see supplementary information of **Study 5**).

These results indicate that chiral monoterpenes are not synthesised *de novo* from primary metabolites inside of roots. This is surprising, since earlier studies have indicated *de novo* synthesis of monoterpenes in roots: Zhou & Pichersky (2020b) identified several mTPS in roots of tomatoes and Chen *et al.* (2004) demonstrated that 1,8-cineole is produced and emitted but not accumulated in roots of *Arabidopsis*. Biosynthesis and emission might be driven by the root cortex and surrounding mucilage (Lin *et al.*, 2007). However, monoterpene biosynthesis could be supplied by more complex intermediates from the shoot (Burlat *et al.*, 2004) rather than pyruvate.

Neither storage pools of needles, nor roots contained detectable amounts of ^{13}C into chiral monoterpenes after labelling with ^{13}C2-pyruvate (**Study 5**). It is not surprising that root storage pools did not incorporate any label, since root emissions of chiral monoterpenes were not labelled as well. However, it is likely that storage pools of needles

are recharged by *de novo* synthesis under unstressed conditions. It is possible that the intensity of applied label was not sufficient to integrate detectable amounts of ^{13}C into storage pools throughout the short period of the experiments. Needle emissions of chiral monoterpenes per h were in the magnitude of 0.045-0.1‰ of needle storage pool size (**Study 5**). Hence, integrated ^{13}C was likely diluted in storage pools of needles below detection limits.

Biosynthesis of different monoterpenes is fuelled by a common precursor pool of GDP. Hence, labelling patterns among *de novo* synthesised chiral monoterpenes should be similar. Surprisingly, labelling patterns among different chiral monoterpenes emitted by Norway spruce varied depending on the isotopic tracer that was applied (**Study 4**). Based on these patterns, the 10 chiral monoterpenes were assigned to three different groups by agglomerative hierarchical clustering: *(i)* storage derived compounds ((-)-β-phellandrene, (-)- and (+)-β-pinene; *(ii)* compounds dominantly labelled via $^{13}C2$-pyruvate ((-)-and (+)-limonene, (-)-α-pinene); and *(iii)* compounds dominantly labelled via $^{13}CO_2$-fumigation ((+)-β-phellandrene, (+)-α-pinene, sabinene, myrcene) (**Study 4**). Notably, the grouping matched product groups of mTPS previously identified in Norway spruce (**Study 4**). My results indicate that biosynthesis and emission of chiral monoterpenes may be compartmented in needles of Norway spruce. Hence, biosynthesis and emission of chiral monoterpenes seems to be regulated in the three different clusters in Norway spruce. Multiple mechanisms could explain why labelling patterns differ among *de novo* synthesised chiral monoterpenes: Chiral monoterpenes dominantly labelled via $^{13}CO_2$ (group *iii*) are likely directly synthesised within plastids of mesophyll cells of needles (**Figure 2**). In contrast, but in accordance with my findings in **Study 3**, monoterpenes that were mainly labelled via $^{13}C2$-pyruvate (group *ii*) could be partially synthesised in the cytosol as by-products of sesquiterpene synthases (Davidovich-Rikanati *et al.*, 2008; Gutensohn *et al.*, 2013) or via enzymes of the Nudix hydrolase family (Magnard *et al.*, 2015; Liu *et al.*, 2018). Alternatively, FDP could be transported to plastids (Bick & Lange, 2003) and utilised by multi-substrate mTPS of the TPS-f-subfamily to form these monoterpenes (Wu *et al.*, 2006; Ruiz-Sola *et al.*, 2016; Dhandapani *et al.*, 2020) (**Figure 2**). Biosynthesis of

monoterpenes might also be sub-compartmented within plastids, analogue to the soluble (stromal) and thylakoid-associated IspS in willow leaves (Wildermuth & Fall, 1996, 1998). Lastly, mTPS synthesising monoterpenes of group *(ii*) could be located in plastids of parenchyma cells (Niinemets & Reichstein, 2002) or leucoplasts surrounding the resin channels (Schürmann *et al.*, 1993; Trapp & Croteau, 2001). Due to a lack of chloroplasts, these cells depend on external metabolites, such as cytosolic pyruvate. Though speculative, these dynamics could explain the observed labelling patterns.

These results show that *de novo* synthesis and emission of chiral monoterpenes is supplied by cytosolic pyruvate in needles, but not in roots of Norway spruce. Above-ground emitted monoterpenes were clustered in three different groups based on their labelling patterns, indicating that chiral monoterpene biosynthesis is driven by different metabolic pathways and likely takes place in multiple compartments. In response to drought, *de novo* synthesis declined and remaining emissions were maintained by storage pools (Study 4 and Study 5).

2.3. Above- and below-ground differences in chiral monoterpene composition imply information on tissue-specific functioning

*Differences in chiral monoterpene composition and concentration of emissions and storage pools above- and below-ground from Norway spruce were analysed before and during drought in **Study 4** and **Study 5**.*

Total concentration and chiral composition significantly differed between needles and roots of Norway spruce (**Study 5**). Needle emissions were 3 times higher than emissions of roots and storage pools were ten times larger in needles compared to roots. In needle emissions and content, (-)-limonene was the dominant monoterpene, whereas (-)-enantiomers of α-pinene, β-pinene and β-phellandrene were predominant in roots (**Study 5**). While enantiomeric ratios were more diverse but generally dominated by the (-)-enantiomers in needles, root enantiomeric ratios where heavily dominated by (-)-enantiomers, except for limonene enantiomers (**Study 5**).

By accounting for all compounds with demonstrated antimicrobial and/or insecticidal properties (both enantiomers of limonene, (+)-α-pinene and (+)-β-pinene, see **chapter 1.3**), contribution of deterring compounds increased during drought from 34% to 50% in needle emissions and from 26% to 32% in storage pools (**Study 5**). In contrast, contribution of these monoterpenes tendentially diminished from 15% to 8% in root emissions and was unaffected in root storage pools (7%). Hence, insecticidal/antimicrobial activity of emitted and stored monoterpenes might be of higher importance above- than below-ground. In parallel, OH reactivity of needle emissions increased in response to drought (**Study 4**). Hence, the controlled increase in deterring and reactive emissions as a consequence to drought might reflect an optimised protection when overall emissions diminished. My results indicate, that insecticidal and antimicrobial activity might be of minor importance in below-ground chiral monoterpene emissions. Roots exude a suite of non-volatile chemicals, many of which are attributed with strong antimicrobial properties (Baetz & Martinoia, 2014). Thus, olfactory cues of chiral monoterpenes might be rather utilised as signalling molecules for mutualistic interactions in below-ground tissue (Rasmann *et al.*, 2005; Ali *et al.*, 2010; Ditengou *et al.*, 2015).

Composition of chiral monoterpenes in needles is dominated by compounds with antimicrobial and/or insecticidal properties and their contribution increases in response to drought, whereas these monoterpenes are of minor abundance in roots, where drought has no effect on chiral monoterpene composition. Consequently, below-ground emissions of chiral monoterpenes might be rather utilised as signalling molecules for beneficial interactions, while deterring functions are more relevant in above-ground emissions. The drought-mediated compositional changes observed in needles could reflect an optimisation of protective properties under declining emissions (Study 4 and Study 5).

2.4. Chiral monoterpene emissions are regulated independent of storage pool size

*One aim of **Study 5** was to investigate if drought affects the dynamics and interactions of chiral monoterpene emissions and storage pools from needles and roots of Norway spruce.*

Total chiral monoterpene emissions declined to one third of control levels in response to drought in both, needles and roots (**Study 5**). Conversely, needle and root storage pools increased by a factor of 1.5 and 2.3, respectively. Hence, the portion of monoterpenes emitted per h relative to monoterpene content in storage pools declined from control (needles: 0.1‰; roots: 0.35‰) to drought (needles: 0.045‰; roots: 0.05‰;) conditions (**Study 5**). *De novo* emissions declined during drought (**Study 4**), thus remaining emissions were driven by storage pools. With about 30% of monoterpene emissions by needles from Norway spruce originating from *de novo* synthesis in undisturbed conditions (Ghirardo *et al.*, 2010), the observed decline in *de novo* synthesis can only partially explain the drop in monoterpene emissions by more than 60% in response to drought. Therefore, emissions from storage pools also declined during drought conditions. Stored monoterpenes are generally seen to be passively emitted from storage compartments in dependence to temperature, even in absence of *de novo* synthesis (Gershenzon *et al.*, 2000; Fischbach *et al.*, 2002). Under long term stress, emissions cannot be sustained from non-

specific storage of monoterpenes in mesophyll cells (Niinemets & Reichstein, 2002). Consequently, monoterpene emissions rely on release and transport from resin ducts and associated cells under drought. Notably, monoterpene emissions are not controlled by stomatal opening as long as monoterpene concentration in the foliage intercellular air space are maintained. Based on my findings, I conclude that the emission of monoterpenes from storage compartments is not solely determined by temperature and storage pool size. Rather, transport processes or release mechanisms seem to control monoterpene emissions from storage pools. These findings are important for emission development in future climate scenarios, as exponential increases of monoterpene emissions in response to rising temperatures (Guenther *et al.*, 2012) could indeed be buffered by the active control of emissions resulting from drought stress.

When emissions from *de novo* synthesis decline, composition and concentration should reflect those of storage compartments. However, my results indicate discrepancies between content and emissions from these compartments. In contrast to the general assumptions that the light-independent part of monoterpene emissions is passively emitted in dependence to temperature, I conclude that additional release or transport processes control the emissions of stored compounds in response to drought. These findings imply important ramifications for modelling approaches, highlighting the uncertainties of current estimates of temperate and boreal monoterpene emissions in a changing climate (Study 5).

3. Conclusion and outlook

3.1. Work in progress and future directions

Chirality is a fundamental phenomenon in biology, observable from macrobiological structures to the molecular level. Even though many biologic processes rely on homochirality, in some compound classes both enantiomers play vital and very different roles for organismic functioning (Bentley, 2006a). The results of this thesis provide evidence that biosynthesis and emissions of monoterpene enantiomers in plants can be driven by different metabolic pathways, which are unevenly affected by drought. Possible ramifications of shifts in chiral composition for plant-environmental interactions could be immense (Lehmanski *et al.*, 2023), but remain speculative. A better understanding of the underlying mechanisms driving chiral product ratios is also of interest for the pharmaceutical and food industry, since biological properties can heavily vary between the pure enantiomers and racemic mixtures (Bentley, 2006a, 2006b; van Vuuren & Viljoen, 2007; Vickers *et al.*, 2014). External application, or direct biosynthesis of specific herbivore-deterring or parasitoid-attracting mixtures of chiral monoterpenes by genetically modified crops could help to improve pest control (Loreto *et al.*, 2014).

In the future, currently available information on compositional changes in chiral monoterpenes in response to environmental stressors should be exploited in above- and below-ground plant-insect interaction studies, to better understand primary attraction of herbivores by stressed tree individuals. Further, antimicrobial and OH-scavenging properties of complex, chiral monoterpene mixtures of stressed and unstressed trees should be analysed to estimate possible protective optimisation in response to environmental stressors. These dynamics should be investigated in plant species of different ecosystems that are prone to pests and extreme climatic events. Notably, more studies should focus on tropical plant species, as they are potentially one of the major sources for chiral monoterpenes (Guenther *et al.*, 2012). As the next step, sophisticated multi-stress and priming experiments (for example, see Kleist *et al.*, 2012; Copolovici *et al.*, 2014; Birami *et al.*, 2021), combining drought, heat and/or herbivore feeding with isotopic labelling will be

necessary to understand the complex interplay of multivariate stressors that plants are faced in natural ecosystems. To this end, more multi-stress experiments on mature trees in natural ecosystems, similar to the study by Trowbridge *et al.* (2021), will be needed to test whether or not laboratory projections are applicable.

To accurately estimate ecosystem fluxes of isoprenoids in a changing climate, contributions of all ecosystem levels need to be assessed. For evaluating changes in source contributions from soil microbes, roots, or foliage to atmospheric concentrations, possible source-specific chiral monoterpene ratios could be exploited (Zannoni *et al.*, 2020; Byron *et al.*, 2022). Especially below-ground contributions remain challenging to estimate, since they are driven by diverse biologic (roots, microbes) and physical (soil moisture & soil properties) processes. Disentangling of, and understanding the interdependencies between these processes will remain a major challenge for future research. Isolated uptake/emission experiments with BVOC standards or natural BVOC mixtures on sterile or artificial soils could help to better understand the interplay between these factors.

The results of this thesis demonstrate the complexity of processes driving isoprenoid biosynthesis in different plant species. Biosynthetic pathways cannot only differ for isoprene and monoterpene biosynthesis, but even for different, chiral monoterpenes that supposedly resort to the same metabolic precursor pool. These findings contribute to a growing amount of evidence that isoprenoid biosynthesis is not separated by different cell compartments (Dudareva *et al.*, 2005; Magnard *et al.*, 2015; Liu *et al.*, 2018; Dhandapani *et al.*, 2020; Zhou & Pichersky, 2020a). I was able to identify these differences by using position-specifically labelled pyruvate. However, the flexibility in metabolic utilisation of pyruvate reduces the certainty for exact tracing of the according pathways that lead to the synthesis of a specific molecule. Additionally, refixation of decarboxylated ^{13}C from $^{13}C1$-pyruvate has been discussed as a possible issue in correctly interpreting pyruvate utilisation (**Study 3**, Fasbender *et al.*, 2018; Kreuzwieser *et al.*, 2021). To better understand the driving forces behind the observed compartmentation in chiral monoterpene synthesis, subsequent studies should aim to disentangle compartment-specific (plastid/cytosol) biosynthesis of different monoterpenes, for example by applying

isotopically labelled, MVA-/MEP-specific precursors for monoterpene biosynthesis. Similar experiments could be conducted to enhance our, currently rudimental understanding of below-ground monoterpene biosynthesis (Chen *et al.*, 2004; Zhou & Pichersky, 2020b), especially for species with specialised storage compartments. However, proper interpretation of these experiments requires standardised conditions. Hence, the conducted and proposed isotopic labelling experiments are in general limited to laboratory conditions. Therefore, the development of proper tools for the investigation of biosynthetic controls, driving metabolic processes of mature trees in response to drought, will remain a major challenge for future research in this field.

3.2. Conclusions

The aim of this thesis was to further our understanding of how above- and below-ground emission and biosynthesis of isoprenoids is regulated in plants in response to drought. Biosynthesis of isoprenoids is highly flexible and can be fuelled by different biosynthetic pathways. Whether or not isoprenoid emissions are maintained during drought, depends on plant species and drought severity. While monoterpene emissions can be partially maintained during drought by increased utilisation of alternative, cytosolic carbon sources, severe drought stress generally leads to strong declines in emissions and uncouples passive emissions from storage pools. Drastic declines in isoprenoid emissions in response to severe drought likely impact plant-environmental interactions (Lehmanski *et al.*, 2023) and reduce resilience against oxidative stress (Sharkey *et al.*, 2008). Shifts in composition of chiral monoterpene emissions might enhance protective potential (Turlings *et al.*, 1990; Loreto *et al.*, 2014), but could also be utilised as cues by herbivores for primary attraction (Erbilgin *et al.*, 2007; Schiebe, 2012). The interacting effects of more frequent, longer summer droughts in a hotter future climate (Spinoni *et al.*, 2020) and increased tree mortality (Allen *et al.*, 2015) on global isoprenoid emissions are still challenging to predict (Loreto & Schnitzler, 2010). Exponential increases in emissions due to rising temperatures (Guenther *et al.*, 2012) might be buffered to a certain extent by emission reductions in response to severe drought (Birami *et al.*, 2021). While increased tree

mortality will likely reduce isoprenoid emissions in broadleaf-dominated tropical (Yáñez-Serrano *et al.*, 2020) and Mediterranean (Pasquini *et al.*, 2023) forests, emission bursts of dying conifer trees (Birami *et al.*, 2021) could significantly increase the contribution of boreal forests to atmospheric isoprenoid concentrations.

In conclusion, this thesis unravelled new insights into above- and below-ground isoprene and chiral monoterpene biosynthesis and emission changes in response to drought. Nonetheless, several questions arise from my experiments, for example regarding above-ground compartmentation of monoterpene biosynthesis, below-ground monoterpene biosynthesis, and regulation of monoterpene emissions from storage compartments. Hence, this thesis may provide the initial steppingstones for further investigations at the frontier of our current understanding of plant isoprenoid biosynthesis and emission regulation.

4. References

Aggarwal KK, Khanuja SPS, Ahmad A, Santha Kumar TR, Gupta VK, Kumar S. 2002. Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicata* and *Anethum sowa*. *Flavour and Fragrance Journal* **17**: 59–63.

Ali JG, Alborn HT, Stelinski LL. 2010. Subterranean herbivore-induced volatiles released by *citrus* roots upon feeding by *Diaprepes abbreviatus* recruit entomopathogenic nematodes. *Journal of Chemical Ecology* **36**: 361–368.

Allen CD, Breshears DD, McDowell NG. 2015. On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. *Ecosphere* **6**: 1-55.

Allen J, Nelson M. 1999. Overview and Design Biospherics and Biosphere 2, mission one (1991–1993). *Ecological Engineering* **13**: 15–29.

Ananyev G, Kolber ZS, Klimov D, Falkowski PG, Berry JA, Rascher U, Martin R, Osmond B. 2005. Remote sensing of heterogeneity in photosynthetic efficiency, electron transport and dissipation of excess light in *Populus deltoides* stands under ambient and elevated CO_2 concentrations, and in a tropical forest canopy, using a new laser-induced fluorescence transient device. *Global Change Biology* **11**: 1195–1206.

Anderegg WRL, Hicke JA, Fisher RA, Allen CD, Aukema J, Bentz BJ, Hood S, Lichstein JW, Macalady AK, McDowell NG *et al.* 2015. Tree mortality from drought, insects, and their interactions in a changing climate. *New Phytologist* **208**: 674–683.

Atkin OK, Macherel D. 2009. The crucial role of plant mitochondria in orchestrating drought tolerance. *Annals of Botany* **103**: 581–597.

Atkinson R, Arey J. 2003. Gas-phase tropospheric chemistry of biogenic volatile organic compounds: a review. *Atmospheric Environment* **37**: 197–219.

Attygalle AB, Wu X, Maddison DR, Will KW. 2009. Orange/lemon-scented beetles: opposite enantiomers of limonene as major constituents in the defensive secretion of related carabids. *Naturwissenschaften* **96**: 1443–1449.

Aubourg S, Lecharny A, Bohlmann J. 2002. Genomic analysis of the terpenoid synthase (AtTPS) gene family of *Arabidopsis thaliana*. *Molecular genetics and genomics* **267**: 730–745.

Baetz U, Martinoia E. 2014. Root exudates: the hidden part of plant defense. *Trends in Plant Science* **19**: 90–98.

Baldwin IT, Halitschke R, Paschold A, von Dahl CC, Preston CA. 2006. Volatile signaling in plant-plant interactions: "talking trees" in the genomics era. *Science* **311**: 812–815.

Barba J, Bradford MA, Brewer PE, Bruhn D, Covey K, van Haren JLM, Megonigal JP, Mikkelsen TN, Pangala SR, Pihlatie M *et al.* 2019. Methane emissions from tree stems: a new frontier in the global carbon cycle. *New Phytologist* **222**: 18–28.

Barron-Gafford GA, Pavao-Zuckerman MA, Minor RL, Sutter LF, Barnett-Moreno I, Blackett DT, Thompson M, Dimond K, Gerlak AK, Nabhan GP *et al.* 2019. Agrivoltaics provide mutual benefits across the food–energy–water nexus in drylands. *Nature Sustainability* **2**: 848–855.

Bartoli CG, Gómez F, Martínez DE, Guiamet JJ. 2004. Mitochondria are the main target for oxidative damage in leaves of wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* **55**: 1663–1669.

Bartram S, Jux A, Gleixner G, Boland W. 2006. Dynamic pathway allocation in early terpenoid biosynthesis of stress-induced lima bean leaves. *Phytochemistry* **67**: 1661–1672.

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

Bentley R. 2006a. Chirality in Biology. *Reviews in Cell Biology and Molecular Medicine*. doi: 10.1002/3527600906.mcb.200200008.

Bentley R. 2006b. The nose as a stereochemist. Enantiomers and odor. *Chemical Reviews* **106**: 4099–4112.

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.

Billings RF, Cameron RS. 1984. Kairomonal Responses of Coleoptera, *Monochamus titillator* (Cerambycidae), *Thanasimus dubius* (Cleridae), and *Temnochila virescens* (Trogositidae), to Behavioral Chemicals of Southern Pine Bark Beetles (Coleoptera: Scolytidae). *Environmental Entomology* **13**: 1542–1548.

Birami B, Bamberger I, Ghirardo A, Grote R, Arneth A, Gaona-Colmán E, Nadal-Sala D, Ruehr NK. 2021. Heatwave frequency and seedling death alter stress-specific emissions of volatile organic compounds in Aleppo pine. *Oecologia* **197**: 939–956.

Borg-Karlson AK, Lindström M, Norin T, Persson M, Valterová I. 1993. Enantiomeric composition of monoterpene hydrocarbons in different tissues of Norway Spruce, *Picea abies* (L) Karst. A multi-dimensional gas chromatography study. *Acta Chemica Scandinavia* **47**: 138–144.

Bougas L, Byron J, Budker D, Williams J. 2022. Absolute optical chiral analysis using cavity-enhanced polarimetry. *Science Advances* **8**: eabm3749.

Bourtsoukidis E, Williams J, Kesselmeier J, Jacobi S, Bonn B. 2014. From emissions to ambient mixing ratios: online seasonal field measurements of volatile organic compounds over a Norway spruce-dominated forest in central Germany. *Atmospheric Chemistry and Physics* **14**: 6495–6510.

Brilli F, Barta C, Fortunati A, Lerdau MT, 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.

Burlat V, Oudin A, Courtois M, Rideau M, St-Pierre B. 2004. Co-expression of three MEP pathway genes and geraniol 10-hydroxylase in internal phloem parenchyma of *Catharanthus roseus* implicates multicellular translocation of intermediates during the biosynthesis of monoterpene indole alkaloids and isoprenoid-derived primary metabolites. *The Plant Journal* **38**: 131–141.

Byron J, Kreuzwieser J, Purser G, van Haren JLM, Ladd SN, Meredith LK, Werner C, Williams J. 2022. Chiral monoterpenes reveal forest emission mechanisms and drought responses. *Nature* **609**: 307–312.

Cabane M, Afif D, Hawkins S. 2012. Chapter 7 - Lignins and Abiotic Stresses. In: *Lignins - Biosynthesis, Biodegradation and Bioengineering*. Elsevier, 219–262.

Capelli L, Sironi S, Del Rosso R. 2013. Odor sampling: techniques and strategies for the estimation of odor emission rates from different source types. *Sensors* **13**: 938–955.

Chen F, Ro DK, Petri J, Gershenzon J, Bohlmann J, Pichersky E, Tholl D. 2004. Characterization of a root-specific *Arabidopsis* terpene synthase responsible for the formation of the volatile monoterpene 1,8-cineole. *Plant Physiology* **135**: 1956–1966.

Chen F, Tholl D, Bohlmann J, Pichersky E. 2011a. The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. *The Plant Journal* **66**: 212–229.

Chen X, Hopke PK, Carter WPL. 2011b. Secondary organic aerosol from ozonolysis of biogenic volatile organic compounds: chamber studies of particle and reactive oxygen species formation. *Environmental Science & Technology* **45**: 276–282.

Cheniclet C, Carde JP. 1985. Presence of leucoplasts in secretory cells and of monoterpenes in the essential oil: a correlative study. *Israel Journal of Plant Sciences* **34**: 219–238.

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.

Cohen JE, Tilman D. 1996. Biosphere 2 and biodiversity: the lessons so far. *Science* **274**: 1150–1151.

Copolovici LO, Filella I, Llusià J, Niinemets Ü, Peñuelas J. 2005. The capacity for thermal protection of photosynthetic electron transport varies for different monoterpenes in *Quercus ilex*. *Plant Physiology* **139**: 485–496.

Copolovici LO, Kännaste A, Remmel T, Niinemets Ü. 2014. Volatile organic compound emissions from Alnus glutinosa under interacting drought and herbivory stresses. *Environmental and Experimental Botany* **100**: 55–63.

Da Rivas Silva AC, Lopes PM, Barros de Azevedo MM, Costa DCM, Alviano CS, Alviano DS. 2012. Biological activities of α-pinene and β-pinene enantiomers. *Molecules* **17**: 6305–6316.

Davidovich-Rikanati R, Lewinsohn E, Bar E, Iijima Y, Pichersky E, Sitrit Y. 2008. Overexpression of the lemon basil alpha-zingiberene synthase gene increases both mono- and sesquiterpene contents in tomato fruit. *The Plant Journal* **56**: 228–238.

de los Santos ZA, Wolf C. 2020. Optical Terpene and Terpenoid Sensing: Chiral Recognition, Determination of Enantiomeric Composition and Total Concentration Analysis with Late Transition Metal Complexes. *Journal of the American Chemical Society* **142**: 4121–4125.

de Souza VF, Niinemets Ü, Rasulov B, Vickers CE, Duvoisin Júnior S, Araújo WL, Gonçalves JFdC. 2018. Alternative carbon sources for isoprene emission. *Trends in Plant Science* **23**: 1081–1101.

Dhandapani S, Tjhang JG, Jang IC. 2020. Production of multiple terpenes of different chain lengths by subcellular targeting of multi-substrate terpene synthase in plants. *Metabolic Engineering* **61**: 397–405.

Diamond J. 1986. Laboratory experiments, field experiments, and natural experiments. *Community ecology*: 3–22.

Ditengou FA, Müller A, Rosenkranz M, Felten J, Lasok H, van Doorn MM, Legué V, Palme K, Schnitzler JP, Polle A. 2015. Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprogrammes root architecture. *Nature Communications* **6**: 6279.

Donath J, Boland W. 1995. Biosynthesis of acyclic homoterpenes: Enzyme selectivity and absolute configuration of the nerolidol precursor. *Phytochemistry* **39**: 785–790.

Dudareva N, Andersson S, Orlova I, Gatto N, Reichelt M, Rhodes D, Boland W, Gershenzon J. 2005. The nonmevalonate pathway supports both monoterpene and sesquiterpene formation in snapdragon flowers. *Proceedings of the National Academy of Sciences* **102**: 933–938.

Dudareva N, Klempien A, Muhlemann JK, Kaplan I. 2013. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytologist* **198**: 16–32.

Dudareva N, Martin DM, Kish CM, Kolosova N, Gorenstein N, Fäldt J, Miller B, Bohlmann J. 2003. (E)-\beta-Ocimene and myrcene synthase genes of floral scent biosynthesis in snapdragon: function and expression of three terpene synthase genes of a new terpene synthase subfamily. *The Plant Cell* **15**: 1227–1241.

Dudareva N, Negre F, Nagegowda DA, Orlova I. 2006. Plant volatiles: recent advances and future perspectives. *Critical Reviews in Plant Sciences* **25**: 417–440.

Dudareva N, Pichersky E, Gershenzon J. 2004. Biochemistry of plant volatiles. *Plant Physiology* **135**: 1893–1902.

Erbilgin N, Krokene P, Kvamme T, Christiansen E. 2007. A host monoterpene influences *Ips typographus* (Coleoptera: Curculionidae, Scolytinae) responses to its aggregation pheromone. *Agricultural and Forest Entomology* **9**: 135–140.

Fasbender L, Yáñez-Serrano AM, Kreuzwieser J, Dubbert D, Werner C. 2018. Real-time carbon allocation into biogenic volatile organic compounds (BVOCs) and respiratory carbon dioxide (CO_2) traced by PTR-TOF-MS, $^{13}CO_2$ laser spectroscopy and ^{13}C-pyruvate labelling. *PloS One* **13**: e0204398.

Fischbach RJ, Staudt M, Zimmer I, Rambal S, Schnitzler JP. 2002. Seasonal pattern of monoterpene synthase activities in leaves of the evergreen tree *Quercus ilex*. *Physiologia Plantarum* **114**: 354–360.

Fischbach RJ, Zimmer W, Schnitzler JP. 2001. Isolation and functional analysis of a cDNA encoding a myrcene synthase from holm oak (*Quercus ilex* L.). *European Journal of Biochemistry* **268**: 5633–5638.

Fortunati A, Barta C, Brilli F, Centritto M, Zimmer I, Schnitzler JP, 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.

Gershenzon J, McConkey ME, Croteau RB. 2000. Regulation of monoterpene accumulation in leaves of peppermint. *Plant Physiology* **122**: 205–214.

Ghimire RP, Kivimäenpää M, Blomqvist M, Holopainen T, Lyytikäinen-Saarenmaa P, Holopainen JK. 2016. Effect of bark beetle (*Ips typographus* L.) attack on bark VOC emissions of Norway spruce (*Picea abies* Karst.) trees. *Atmospheric Environment* **126**: 145–152.

Ghirardo A, Gutknecht J, Zimmer I, Brüggemann N, Schnitzler JP. 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.

Ghirardo A, Koch K, Taipale R, Zimmer I, Schnitzler JP, Rinne J. 2010. Determination of *de novo* and pool emissions of terpenes from four common boreal/alpine trees by $^{13}CO_2$ labelling and PTR-MS analysis. *Plant, Cell & Environment* **33**: 781–792.

Grossiord C, Granier A, Gessler A, Jucker T, Bonal D. 2014. Does Drought Influence the Relationship Between Biodiversity and Ecosystem Functioning in Boreal Forests? *Ecosystems* **17**: 394–404.

Grote R, Lavoir AV, Rambal S, Staudt M, Zimmer I, Schnitzler JP. 2009. Modelling the drought impact on monoterpene fluxes from an evergreen Mediterranean forest canopy. *Oecologia* **160**: 213–223.

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

Guenther AB, Jiang X, Heald CL, Sakulyanontvittaya T, Duhl T, Emmons LK, Wang X. 2012. The Model of Emissions of Gases and Aerosols from Nature version 2.1 (MEGAN2. 1): an extended and updated framework for modeling biogenic emissions. *Geoscientific Model Development* **5**: 1471–1492.

Guenther AB, Monson RK, Fall R. 1991. Isoprene and monoterpene emission rate variability: Observations with eucalyptus and emission rate algorithm development. *Journal of Geophysical Research* **96**: 10799–10808.

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.

Gutensohn M, Orlova I, Nguyen TTH, Davidovich-Rikanati R, Ferruzzi MG, Sitrit Y, Lewinsohn E, Pichersky E, Dudareva N. 2013. Cytosolic monoterpene biosynthesis is supported by plastid-generated geranyl diphosphate substrate in transgenic tomato fruits. *The Plant Journal* **75**: 351–363.

Hanning I, Heldt HW. 1993. On the Function of Mitochondrial Metabolism during Photosynthesis in Spinach (*Spinacia oleracea* L.) Leaves (Partitioning between Respiration and Export of Redox Equivalents and Precursors for Nitrate Assimilation Products). *Plant Physiology* **103**: 1147–1154.

Hantson S, Knorr W, Schurgers G, Pugh TA, Arneth A. 2017. Global isoprene and monoterpene emissions under changing climate, vegetation, CO_2 and land use. *Atmospheric Environment* **155**: 35–45.

Hartmann H, Bahn M, Carbone M, Richardson AD. 2020. Plant carbon allocation in a changing world - challenges and progress: Introduction to a virtual issue on carbon allocation. *New Phytologist* **227**: 981–988.

Hiltpold I, Turlings TCJ. 2012. Manipulation of chemically mediated interactions in agricultural soils to enhance the control of crop pests and to improve crop yield. *Journal of Chemical Ecology* **38**: 641–650.

Hoefnagel MHN, Atkin OK, Wiskich JT. 1998. Interdependence between chloroplasts and mitochondria in the light and the dark. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1366**: 235–255.

Holopainen JK, Gershenzon J. 2010. Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science* **15**: 176–184.

Holzinger R. 2015. PTRwid: A new widget tool for processing PTR-TOF-MS data. *Atmospheric Measurement Techniques* **8**: 3903–3922.

Holzinger R, Acton WJF, Bloss WJ, Breitenlechner M, Crilley LR, Dusanter S, Gonin M, Gros V, Keutsch FN, Kiendler-Scharr A *et al.* 2019. Validity and limitations of simple reaction kinetics to calculate concentrations of organic compounds from ion counts in PTR-MS. *Atmospheric Measurement Techniques* **12**: 6193–6208.

Honeker LK, Pugliese G, Ingrisch J, Fudyma JD, Gil-Loaiza J, Carpenter E, Singer E, Hildebrand GA, Shi L, Daber LE *et al.* 2022. Effect of drought on soil microbial metabolisms driving carbon allocation and volatile organic compound cycling in the tropical rainforest at Biosphere 2. *Authorea Preprints*. doi: 10.1002/essoar.10510038.1.

Hopp L, Harman C, Desilets SLE, Graham CB, McDonnell JJ, Troch PA. 2009. Hillslope hydrology under glass: confronting fundamental questions of soil-water-biota co-evolution at Biosphere 2. *Hydrology and Earth System Sciences* **13**: 2105–2118.

Huang M, Sanchez-Moreiras AM, Abel C, Sohrabi R, Lee S, Gershenzon J, Tholl D. 2012. The major volatile organic compound emitted from *Arabidopsis thaliana* flowers, the

sesquiterpene (*E*)-β-caryophyllene, is a defense against a bacterial pathogen. *New Phytologist* **193**: 997–1008.

Jardine KJ, Sommer ED, Saleska SR, Huxman TE, Harley PC, Abrell L. 2010. Gas phase measurements of pyruvic acid and its volatile metabolites. *Environmental Science & Technology* **44**: 2454–2460.

Jardine KJ, Wegener F, Abrell L, van Haren JLM, Werner C. 2014. Phytogenic biosynthesis and emission of methyl acetate. *Plant, Cell and Environment* **37**: 414–424.

Jordan A, Haidacher S, Hanel G, Hartungen E, Märk L, Seehauser H, Schottkowsky R, Sulzer P, Märk TD. 2009. A high resolution and high sensitivity proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF-MS). *International Journal of Mass Spectrometry* **286**: 122–128.

Kanakidou M, Seinfeld JH, Pandis SN, Barnes I, Dentener FJ, Facchini MC, van Dingenen R, Ervens B, Nenes A, Nielsen CJ *et al.* 2005. Organic aerosol and global climate modelling: a review. *Atmospheric Chemistry and Physics* **5**: 1053–1123.

Kleist E, Mentel TF, Andres S, Bohne A, Folkers A, Kiendler-Scharr A, Rudich Y, Springer M, Tillmann R, Wildt J. 2012. Irreversible impacts of heat on the emissions of monoterpenes, sesquiterpenes, phenolic BVOC and green leaf volatiles from several tree species. *Biogeosciences* **9**: 5111.

Klimetzek D, Vité JP, Mori K. 1980. Zur Wirkung und Formulierung des Populationslockstoffes des Nutzholzborkenkäfers *Trypodendron* (*Xyloterus*) *lineatum* 1. *Zeitschrift für Angewandte Entomologie* **89**: 57–63.

Kreuzwieser J, Meischner M, Grün M, Yáñez-Serrano AM, Fasbender L, Werner C. 2021. Drought affects carbon partitioning into volatile organic compound biosynthesis in Scots pine needles. *New Phytologist* **232**: 1930–1943.

Kuck D. 2002. Half a century of scrambling in organic ions: complete, incomplete, progressive and composite atom interchange. *International Journal of Mass Spectrometry* **213**: 101–144.

Kumar A, Víden I. 2007. Volatile organic compounds: sampling methods and their worldwide profile in ambient air. *Environmental Monitoring and Assessment* **131**: 301–321.

Kunert G, Otto S, Röse USR, Gershenzon J, Weisser WW. 2005. Alarm pheromone mediates production of winged dispersal morphs in aphids. *Ecology Letters* **8**: 596–603.

Ladd SN, Nelson DB, Bamberger I, Daber LE, Kreuzwieser J, Kahmen A, Werner C. 2021. Metabolic exchange between pathways for isoprenoid synthesis and implications for biosynthetic hydrogen isotope fractionation. *New Phytologist* **231**: 1708–1719.

Lamarque JF, Bond TC, Eyring V, Granier C, Heil A, Klimont Z, Lee D, Liousse C, Mieville A, Owen B *et al.* 2010. Historical (1850–2000) gridded anthropogenic and biomass burning emissions of reactive gases and aerosols: methodology and application. *Atmospheric Chemistry and Physics* **10**: 7017–7039.

Laothawornkitkul J, Taylor JE, Paul ND, Hewitt CN. 2009. Biogenic volatile organic compounds in the Earth system. *New Phytologist* **183**: 27–51.

Lathière J, Hauglustaine DA, Friend AD, de Noblet-Ducoudré N, Viovy N, Folberth GA. 2006. Impact of climate variability and land use changes on global biogenic volatile organic compound emissions. *Atmospheric Chemistry and Physics* **6**: 2129–2146.

Laule O, Fürholz A, Chang HS, Zhu T, Wang X, Heifetz PB, Gruissem W, Lange BM. 2003. Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences* **100**: 6866–6871.

Lavoir AV, Staudt M, Schnitzler JP, Landais D, Massol F, Rocheteau A, Rodriguez R, Zimmer I, Rambal S. 2009. Drought reduced monoterpene emissions from the evergreen Mediterranean oak *Quercus ilex*: results from a throughfall displacement experiment. *Biogeosciences* **6**: 1167–1180.

Lawlor DW, Khanna-Chopra R. 1984. Regulation of Photosynthesis During Water Stress. In: *Advances in Photosynthesis Research: Proceedings of the VIth International Congress on Photosynthesis, Brussels, Belgium, August 1-6, 1983. Volume 4.* Springer, Dordrecht.

Lehmanski LMA, Kandasamy D, Andersson MN, Netherer S, Alves EG, Huang J, Hartmann H. 2023. Addressing a century-old hypothesis - do pioneer beetles of *Ips typographus* use volatile cues to find suitable host trees? *New Phytologist* **238**: 1762–1770.

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 and Environment* **22**: 495–504.

Lelieveld J, Butler TM, Crowley JN, Dillon TJ, Fischer H, Ganzeveld L, Harder H, Lawrence MG, Martinez M, Taraborrelli D *et al.* 2008. Atmospheric oxidation capacity sustained by a tropical forest. *Nature* **452**: 737–740.

Lewinsohn E, Gijzen M, Muzika RM, Barton K, Croteau RB. 1993. Oleoresinosis in Grand Fir (*Abies grandis*) saplings and mature trees (modulation of this wound response by light and water stresses). *Plant Physiology* **101**: 1021–1028.

Lin C, Owen SM, Peñuelas J. 2007. Volatile organic compounds in the roots and rhizosphere of *Pinus* spp. *Soil Biology and Biochemistry* **39**: 951–960.

Lin G, Marino BD, Wei Y, Adams J, Tubiello F, Berry JA. 1998. An experimental and modeling study of responses in ecosystems carbon exchanges to increasing CO_2 concentrations using a tropical rainforest mesocosm. *Functional Plant Biology* **25**: 547–556.

Lindeman LP, Annis JL. 1960. Use of a conventional mass spectrometer as a detector for gas chromatography. *Analytical Chemistry* **32**: 1742–1749.

Lindström M, Norin T, Sjödin K. 1990. Plant constituents as signals for aggregation and attack of pest insects. *Pure and Applied Chemistry* **62**: 1329–1332.

Linskens HF, Jackson JF, eds. 1997. Modern Methods of plant Analysis. Plant Volatile Analysis. Berlin: Springer.

Liu J, Guan Z, Liu H, Qi L, Zhang D, Zou T, Yin P. 2018. Structural insights into the substrate recognition mechanism of *Arabidopsis* GPP-bound NUDX1 for noncanonical monoterpene biosynthesis. *Molecular plant* **11**: 218–221.

Loreto F, Ciccioli P, Brancaleoni E, Cecinato A, Frattoni M. 1998a. Measurement of isoprenoid content in leaves of Mediterranean *Quercus* spp. by a novel and sensitive method and estimation of the isoprenoid partition between liquid and gas phase inside the leaves. *Plant Science* **136**: 25–30.

Loreto F, Dicke M, Schnitzler JP, Turlings TCJ. 2014. Plant volatiles and the environment. *Plant, Cell and Environment* **37**: 1905–1908.

Loreto F, Forster A, Durr M, Csiky O, Seufert G. 1998b. On the monoterpene emission under heat stress and on the increased thermotolerance of leaves of *Quercus ilex* L. fumigated with selected monoterpenes. *Plant, Cell and Environment* **21**: 101–107.

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

Loreto F, Pinelli P, Manes F, Kollist H. 2004. Impact of ozone on monoterpene emissions and evidence for an isoprene-like antioxidant action of monoterpenes emitted by *Quercus ilex* leaves. *Tree Physiology* **24**: 361–367.

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

Lücker J, El Tamer MK, Schwab W, Verstappen FWA, van der Plas LHW, Bouwmeester HJ, Verhoeven HA. 2002. Monoterpene biosynthesis in lemon (*Citrus limon*). cDNA isolation and functional analysis of four monoterpene synthases. *European Journal of Biochemistry* **269**: 3160–3171.

Lüpke M, Leuchner M, Steinbrecher R, Menzel A. 2017. Quantification of monoterpene emission sources of a conifer species in response to experimental drought. *AoB PLANTS* **9**: plx045.

Magnard JL, Roccia A, Caissard JC, Vergne P, Sun P, Hecquet R, Dubois A, Hibrand-Saint Oyant L, Jullien F, Nicolè F *et al.* 2015. Biosynthesis of monoterpene scent compounds in roses. *Science* **349**: 81–83.

Mäntylä E, Alessio GA, Blande JD, Heijari J, Holopainen JK, Laaksonen T, Piirtola P, Klemola T. 2008. From plants to birds: higher avian predation rates in trees responding to insect herbivory. *PLOS ONE* **3**: e2832.

Masson-Delmotte VP, Zhai P, Pirani SL, Connors C, Péan S, Berger N, Caud Y, Chen L, Goldfarb MI, Scheel Monteiro PM. 2021. IPCC, 2021: Summary for Policymakers. In: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.

McCaskill D, Croteau RB. 1995. Monoterpene and sesquiterpene biosynthesis in glandular trichomes of peppermint (*Mentha x piperita*) rely exclusively on plastid-derived isopentenyl diphosphate. *Planta* **197**: 49–56.

Meischner M, Haberstroh S, Daber LE, Kreuzwieser J, Caldeira MC, Schnitzler JP, Werner C. 2022. Soil VOC emissions of a Mediterranean woodland are sensitive to shrub invasion. *Plant Biology* **24**: 967–978.

Messina P, Lathière J, Sindelarova K, Vuichard N, Granier C, Ghattas J, Cozic A, Hauglustaine DA. 2016. Global biogenic volatile organic compound emissions in the ORCHIDEE and MEGAN models and sensitivity to key parameters. *Atmospheric Chemistry and Physics* **16**: 14169–14202.

Mikaili P, Mojaverrostami S, Moloudizargari M, Aghajanshakeri S. 2013. Pharmacological and therapeutic effects of *Mentha Longifolia* L. and its main constituent, menthol. *Ancient Science of Life* **33**: 131–138.

Miloradovic van Doorn M, Merl Pham J, Ghirardo A, Fink S, Polle A, Schnitzler JP, Rosenkranz M. 2020. Root isoprene formation alters lateral root development. *Plant, Cell & Environment* **43**: 2207–2223.

Monson RK, Jaeger CH, Adams III WW, Driggers EM, Silver GM, Fall R. 1992. Relationships among isoprene emission rate, photosynthesis, and isoprene synthase activity as influenced by temperature. *Plant Physiology* **98**: 1175–1180.

Munné-Bosch S, Alegre L. 2004. Die and let live: leaf senescence contributes to plant survival under drought stress. *Functional Plant Biology* **31**: 203–216.

Niinemets Ü, Loreto F, Reichstein M. 2004. Physiological and physicochemical controls on foliar volatile organic compound emissions. *Trends in Plant Science* **9**: 180–186.

Niinemets Ü, Reichstein M. 2002. A model analysis of the effects of nonspecific monoterpenoid storage in leaf tissues on emission kinetics and composition in Mediterranean sclerophyllous *Quercus* species. *Global Biogeochemical Cycles* **16**: 57-1.

Noctor G, Veljovic-Jovanovic S, Driscoll SD, Novitskaya L, Foyer CH. 2002. Drought and oxidative load in the leaves of C3 plants: a predominant role for photorespiration? *Annals of Botany* **89**: 841–850.

Nölscher AC, Bourtsoukidis E, Bonn B, Kesselmeier J, Lelieveld J, Williams J. 2013. Seasonal measurements of total OH reactivity emission rates from Norway spruce in 2011. *Biogeosciences* **10**: 4241–4257.

Norin T. 1996. Chiral chemodiversity and its role for biological activity. Some observations from studies on insect/insect and insect/plant relationships. *Pure and Applied Chemistry* **68**: 2043–2049.

Opitz S, Nes WD, Gershenzon J. 2014. Both methylerythritol phosphate and mevalonate pathways contribute to biosynthesis of each of the major isoprenoid classes in young cotton seedlings. *Phytochemistry* **98**: 110–119.

Pan Y, Birdsey RA, Fang J, Houghton R, Kauppi PE, Kurz WA, Phillips OL, Shvidenko A, Lewis SL, Canadell JG *et al.* 2011. A large and persistent carbon sink in the world's forests. *Science* **333**: 988–993.

Pasquini D, Gori A, Pollastrini M, Alderotti F, Centritto M, Ferrini F, Brunetti C. 2023. Effects of drought-induced holm oak dieback on BVOCs emissions in a Mediterranean forest. *Science of the total environment* **857**: 159635.

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

Peñuelas J, Llusià J. 2002. Linking photorespiration, monoterpenes and thermotolerance in *Quercus*. *New Phytologist* **155**: 227–237.

Peñuelas J, Llusià J. 2003. BVOCs: plant defense against climate warming? *Trends in Plant Science* **8**: 105–109.

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

Persson M, Borg-Karlson AK, Norin T. 1993. Enantiomeric composition of six chiral monoterpene hydrocarbons in different tissues of *Picea abies*. *Phytochemistry* **33**: 303–307.

Phillips MA, Savage TJ, Croteau R. 1999. Monoterpene synthases of loblolly pine (*Pinus taeda*) produce pinene isomers and enantiomers. *Archives of Biochemistry and Biophysics* **372**: 197–204.

Piutti A. 1886. Ein neues Asparagin. *Berichte der deutschen chemischen Gesellschaft* **19**: 1691–1695.

Piva LRdO, Jardine KJ, Gimenez BO, de Oliveira Perdiz R, Menezes VS, Durgante FM, Cobello LO, Higuchi N, Chambers JQ. 2019. Volatile monoterpene 'fingerprints' of resinous *Protium* tree species in the Amazon rainforest. *Phytochemistry* **160**: 61–70.

Powis I, Janssen MHM. 2017. Direct Enantiomer-Selective Mass Spectrometry of Chiral Mixtures by Mass-Selected Photoelectron Circular Dichroism. *LCGC Supplements* **15**: 16–23.

Raguso RA. 2008. Start making scents: the challenge of integrating chemistry into pollination ecology. *Entomologia Experimentalis et Applicata* **128**: 196–207.

Rasmann S, Köllner TG, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings TCJ. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* **434**: 732–737.

Rasulov B, Talts E, Bichele I, Niinemets Ü. 2018. Evidence that isoprene emission is not limited by cytosolic metabolites. Exogenous malate does not invert the reverse sensitivity of isoprene emission to high CO_2. *Plant Physiology* **176**: 1573–1586.

Riccobono F, Schobesberger S, Scott CE, Dommen J, Ortega IK, Rondo L, Almeida J, Amorim A, Bianchi F, Breitenlechner M *et al.* 2014. Oxidation products of biogenic emissions contribute to nucleation of atmospheric particles. *Science* **344**: 717–721.

Riedlmeier M, Ghirardo A, Wenig M, Knappe C, Koch K, Georgii E, Dey S, Parker JE, Schnitzler JP, Vlot AC. 2017. Monoterpenes Support Systemic Acquired Resistance within and between Plants. *The Plant Cell* **29**: 1440–1459.

Roach TNF, Little M, Arts MGI, Huckeba J, Haas AF, George EE, Quinn RA, Cobián-Güemes AG, Naliboff DS, Silveira CB *et al.* 2020. A multiomic analysis of in situ coral-turf algal interactions. *Proceedings of the National Academy of Sciences of the United States of America* **117**: 13588–13595.

Rodríguez-Concepción M, Boronat A. 2015. Breaking new ground in the regulation of the early steps of plant isoprenoid biosynthesis. *Current opinion in plant biology* **25**: 17–22.

Rohmer M. 1999. The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. *Natural Product Reports* **16**: 565–574.

Rohmer M, Knani M, Simonin P, Sutter B, Sahm H. 1993. Isoprenoid biosynthesis in bacteria: a novel pathway for the early steps leading to isopentenyl diphosphate. *Biochemical Journal* **295**: 517–524.

Rosenstiel TN, Ebbets AL, Khatri WC, Fall R, Monson RK. 2004. Induction of poplar leaf nitrate reductase: a test of extrachloroplastic control of isoprene emission rate. *Plant Biology* **6**: 12–21.

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.

Rufino AT, Ribeiro M, Judas F, Salgueiro L, Lopes MC, Cavaleiro C, Mendes AF. 2014. Anti-inflammatory and chondroprotective activity of (+)-α-pinene: structural and enantiomeric selectivity. *Journal of Natural Products* **77**: 264–269.

Ruiz-Sola MÁ, Coman D, Beck G, Barja MV, Colinas M, Graf A, Welsch R, Rütimann P, Bühlmann P, Bigler L *et al.* 2016. *Arabidopsis* geranylgeranyl diphosphate synthase 11 is a hub isozyme required for the production of most photosynthesis-related isoprenoids. *New Phytologist* **209**: 252–264.

Saebo A, Hanslin HM, Baraldi R, Rapparini F, Gawronska H, Gawronski SW. 2013. Characterization of urban trees and shrubs for particulate deposition, carbon sequestration and BVOC emissions. *Acta Horticulturae*: 509–517.

Satoh T, Kubo A, Hazama H, Awazu K, Toyoda M. 2014. Separation of Isobaric Compounds Using a Spiral Orbit Type Time-of-Flight Mass Spectrometer, MALDI-SpiralTOF. *Mass spectrometry* **3**: S0027.

Schiebe C. 2012. Attraction and resistance in the *Picea abies* – *Ips typographus* system. *Acta Universitatis Agriculturae Sueciae* **2012:91**.

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.

Schuhr CA, Radykewicz T, Sagner S, Latzel C, Zenk MH, Arigoni D, Bacher A, Rohdich F, Eisenreich W. 2003. Quantitative assessment of crosstalk between the two isoprenoid biosynthesis pathways in plants by NMR spectroscopy. *Phytochemistry Reviews* **2**: 3–16.

Schulz-Bohm K, Gerards S, Hundscheid M, Melenhorst J, de Boer W, Garbeva P. 2018. Calling from distance: attraction of soil bacteria by plant root volatiles. *The ISME Journal* **12**: 1252–1262.

Schürmann W, Ziegler H, Kotzias D, Schönwitz R, Steinbrecher R. 1993. Emission of biosynthesized monoterpenes from needles of Norway Spruce. *Naturwissenschaften* **80**: 276–278.

Shao M, Czapiewski KV, Heiden AC, Kobel K, Komenda M, Koppmann R, Wildt J. 2001. Volatile organic compound emissions from Scots pine: mechanisms and description by algorithms. *Journal of Geophysical Research: Atmospheres* **106**: 20483–20491.

Sharkey TD, Monson RK. 2014. The future of isoprene emission from leaves, canopies and landscapes. *Plant, Cell and Environment* **37**: 1727–1740.

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 emission from plants. *Annual Review of Plant Biology* **52**: 407–436.

Sindelarova K, Granier C, Bouarar I, Guenther AB, Tilmes S, Stavrakou T, Müller J-F, Kuhn U, Stefani P, Knorr W. 2014. Global data set of biogenic VOC emissions calculated by the MEGAN model over the last 30 years. *Atmospheric Chemistry and Physics* **14**: 9317–9341.

Sindelarova K, Markova J, Simpson D, Huszar P, Karlicky J, Darras S, Granier C. 2022. High-resolution biogenic global emission inventory for the time period 2000–2019 for air quality modelling. *Earth System Science Data* **14**: 251–270.

Singsaas EL, Lerdau MT, Winter K, Sharkey TD. 1997. Isoprene Increases Thermotolerance of Isoprene-Emitting Species. *Plant Physiology* **115**: 1413–1420.

Siwko ME, Marrink SJ, de Vries AH, Kozubek A, Schoot Uiterkamp AJM, Mark AE. 2007. Does isoprene protect plant membranes from thermal shock? A molecular dynamics study. *Biochimica et biophysica acta* **1768**: 198–206.

Sjödin K, Persson M, Borg-Karlson AK, Norin T. 1996. Enantiomeric compositions of monoterpene hydrocarbons in different tissues of four individuals of *Pinus sylvestris*. *Phytochemistry* **41**: 439–445.

Smith MN, Taylor TC, van Haren JLM, Rosolem R, Restrepo-Coupe N, Adams J, Wu J, de Oliveira RC, Da Silva R, de Araujo AC *et al.* 2020. Empirical evidence for resilience of tropical forest photosynthesis in a warmer world. *Nature Plants* **6**: 1225–1230.

Spinoni J, Barbosa P, Bucchignani E, Cassano J, Cavazos T, Christensen JH, Christensen OB, Coppola E, Evans J, Geyer B *et al.* 2020. Future Global Meteorological Drought Hot Spots: A Study Based on CORDEX Data. *Journal of Climate* **33**: 3635–3661.

Spurgeon SL, Porter JW. 1981. Biosynthesis of isoprenoid compounds. New York: Wiley.

Staudt M, Bertin N, Hansen U, Seufert G, Cicciolij P, Foster P, Frenzel B, Fugit JL. 1997. Seasonal and diurnal patterns of monoterpene emissions from *Pinus pinea* (L.) under field conditions. *Atmospheric Environment* **31**: 145–156.

Sweetlove LJ, Beard KFM, Nunes-Nesi A, Fernie AR, Ratcliffe RG. 2010. Not just a circle: flux modes in the plant TCA cycle. *Trends in Plant Science* **15**: 462–470.

Taylor TC, McMahon SM, Smith MN, Boyle B, Violle C, van Haren JLM, Simova I, Meir P, Ferreira LV, de Camargo PB *et al.* 2018. Isoprene emission structures tropical tree biogeography and community assembly responses to climate. *New Phytologist* **220**: 435–446.

Tcherkez G, Gauthier P, Buckley TN, Busch FA, Barbour MM, Bruhn D, Heskel MA, Gong XY, Crous KY, Griffin KL *et al.* 2017. Leaf day respiration: low CO_2 flux but high significance for metabolism and carbon balance. *New Phytologist* **216**: 986–1001.

Tcherkez G, Mahé A, Gauthier P, Mauve C, Gout E, Bligny R, Cornic G, Hodges M. 2009. In folio respiratory fluxomics revealed by ^{13}C isotopic labeling and H/D isotope effects highlight the noncyclic nature of the tricarboxylic acid "cycle" in illuminated leaves. *Plant Physiology* **151**: 620–630.

Tholl D, Boland W, Hansel A, Loreto F, Röse USR, Schnitzler JP. 2006. Practical approaches to plant volatile analysis. *The Plant Journal* **45**: 540–560.

Thornhill G, Collins W, Olivié D, Skeie RB, Archibald A, Bauer S, Checa-Garcia R, Fiedler S, Folberth GA, Gjermundsen A *et al.* 2021. Climate-driven chemistry and aerosol feedbacks in CMIP6 Earth system models. *Atmospheric Chemistry and Physics* **21**: 1105–1126.

Trapp S, Croteau RB. 2001. Defensive resin biosynthesis in conifers. *Annual Review of Plant Biology* **52**: 689–724.

Trowbridge AM, Adams HD, Collins A, Dickman LT, Grossiord C, Hofland M, Malone S, Weaver DK, Sevanto S, Stoy PC *et al.* 2021. Hotter droughts alter resource allocation to chemical defenses in piñon pine. *Oecologia* **197**: 921–938.

Tuet WY, Chen Y, Xu L, Fok S, Gao D, Weber RJ, Ng NL. 2017. Chemical oxidative potential of secondary organic aerosol (SOA) generated from the photooxidation of biogenic and anthropogenic volatile organic compounds. *Atmospheric Chemistry and Physics* **17**: 839–853.

Turlings TCJ, Tumlinson JH, Lewis WJ. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* **250**: 1251–1253.

Unger N. 2014. Human land-use-driven reduction of forest volatiles cools global climate. *Nature Climate Change* **4**: 907–910.

Unsicker SB, Kunert G, Gershenzon J. 2009. Protective perfumes: the role of vegetative volatiles in plant defense against herbivores. *Current opinion in plant biology* **12**: 479–485.

van Haren JLM, Handley LL, Biel KY, Kudeyarov VN, McLain JET, Martens DA, Colodner DC. 2005. Drought-induced nitrous oxide flux dynamics in an enclosed tropical forest. *Global Change Biology* **11**: 1247–1257.

van Vuuren SF, Viljoen AM. 2007. Antimicrobial activity of limonene enantiomers and 1,8-cineole alone and in combination. *Flavour and Fragrance Journal* **22**: 540–544.

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 and Environment* **28**: 318–327.

Vickers CE, Bongers M, Liu Q, Delatte T, Bouwmeester HJ. 2014. Metabolic engineering of volatile isoprenoids in plants and microbes. *Plant, Cell and Environment* **37**: 1753–1775.

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.

Wang Q, Reddy VA, Panicker D, Mao HZ, Kumar N, Rajan C, Venkatesh PN, Chua NH, Sarojam R. 2016. Metabolic engineering of terpene biosynthesis in plants using a trichome-specific transcription factor MsYABBY5 from spearmint (*Mentha spicata*). *Plant Biotechnology Journal* **14**: 1619–1632.

Werner C, Fasbender L, Romek KM, Yáñez-Serrano AM, Kreuzwieser J. 2020. Heat waves change plant carbon allocation among primary and secondary metabolism altering CO_2 assimilation, respiration, and VOC emissions. *Frontiers in Plant Science* **11**: 1242.

Werner C, Meredith LK, Ladd SN, Ingrisch J, Kübert A, van Haren JLM, Bahn M, Bamberger I, Beyer M, Blomdahl D *et al.* 2021. Ecosystem fluxes during drought and recovery in an experimental forest. *Science* **374**: 1514–1518.

Wigneron JP, Fan L, Ciais P, Bastos A, Brandt M, Chave J, Saatchi S, Baccini A, Fensholt R. 2020. Tropical forests did not recover from the strong 2015-2016 El Niño event. *Science Advances* **6**: eaay4603.

Wildermuth MC, Fall R. 1996. Light-dependent isoprene emission (characterization of a thylakoid-bound isoprene synthase in *Salix discolor* Chloroplasts). *Plant Physiology* **112**: 171–182.

Wildermuth MC, Fall R. 1998. Biochemical characterization of stromal and thylakoid-bound Isoforms of isoprene synthase in willow Leaves. *Plant Physiology* **116**: 1111–1123.

Williams J, Crowley JN, Fischer H, Harder H, Martinez M, Petäjä T, Rinne J, Bäck J, Boy M, Dal Maso M *et al.* 2011. The summertime Boreal forest field measurement intensive (HUMPPA-COPEC-2010): an overview of meteorological and chemical influences. *Atmospheric Chemistry and Physics* **11**: 10599–10618.

Wolff MM, Stephens WE. 1953. A Pulsed Mass Spectrometer with Time Dispersion. *Review of Scientific Instruments* **24**: 616–617.

Wu C, Pullinen I, Andres S, Carriero G, Fares S, Goldbach H, Hacker L, Kasal T, Kiendler-Scharr A, Kleist E *et al.* 2015. Impacts of soil moisture on *de novo* monoterpene emissions from European beech, Holm oak, Scots pine, and Norway spruce. *Biogeosciences* **12**: 177–191.

Wu S, Schalk M, Clark A, Miles RB, Coates R, Chappell J. 2006. Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants. *Nature biotechnology* **24**: 1441–1447.

Yáñez-Serrano AM, Bourtsoukidis E, Alves EG, Bauwens M, Stavrakou T, Llusià J, Filella I, Guenther AB, Williams J, Artaxo P *et al.* 2020. Amazonian biogenic volatile organic compounds under global change. *Global Change Biology* **26**: 4722–4751.

Yáñez-Serrano AM, Mahlau L, Fasbender L, Byron J, Williams J, Kreuzwieser J, Werner C. 2019. Heat stress increases the use of cytosolic pyruvate for isoprene biosynthesis. *Journal of Experimental Botany* **70**: 5827–5838.

Yassaa N, Williams J. 2007. Enantiomeric monoterpene emissions from natural and damaged Scots pine in a boreal coniferous forest measured using solid-phase microextraction and gas chromatography/mass spectrometry. *Journal of Chromatography A* **1141**: 138–144.

Zannoni N, Leppla D, Lembo Silveira de Assis, Pedro Ivo, Hoffmann T, Sá MO, Araújo A, Williams J. 2020. Surprising chiral composition changes over the Amazon rainforest with height, time and season. *Communications Earth & Environment* **1**: 1–11.

Zhou F, Pichersky E. 2020a. More is better: the diversity of terpene metabolism in plants. *Current opinion in plant biology* **55**: 1–10.

Zhou F, Pichersky E. 2020b. The complete functional characterisation of the terpene synthase family in tomato. *New Phytologist* **226**: 1341–1360.

Zia R, Nawaz MS, Siddique MJ, Hakim S, Imran A. 2021. Plant survival under drought stress: Implications, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. *Microbiological Research* **242**: 126626.

5. Study overview and specific contribution

Study 1

Ecosystem fluxes during drought and recovery in an experimental forest.

Christiane Werner, Laura K. Meredith, S. Nemiah Ladd, Johannes Ingrisch, Angelika Kübert, Joost van Haren, Michael Bahn, Kinzie Bailey, Ines Bamberger, Matthias Beyer, Daniel Blomdahl, Joseph Byron, **L. Erik Daber**, Jason Deleeuw, Michaela A. Dippold, Jane Fudyma, Juliana Gil-Loaiza, Linnea K. Honeker, Jia Hu, Jianbei Huang, Thomas Klüpfel, Jordan Krechmer, Jürgen Kreuzwieser, Kathrin Kühnhammer, Marco M. Lehmann, Kathiravan Meeran, Pawel K. Misztal, Wei-Ren Ng, Eva Pfannerstill, Giovanni Pugliese, Gemma Purser, Joseph Roscioli, Lingling Shi, Malak Tfaily & Jonathan Williams

Status: published in ***Science*** in 2021, doi: 10.1126/science.abj6789

Contribution (%)	Study design	Data collection	Data analysis	Discussions	Writing
L. Erik Daber	0	7	5	5	5

For a detailed contribution list, see:
https://arizona.figshare.com/articles/dataset/B2WALD_Campaign_Team_and_Contributions/14632662?file=31021090

Study 2

Elucidating Drought-Tolerance Mechanisms in Plant Roots through ^{1}H NMR Metabolomics in Parallel with MALDI-MS, and NanoSIMS Imaging Techniques

Linnea K. Honeker, Gina A. Hildebrand, Jane D. Fudyma, **L. Erik Daber**, David Hoyt, Sarah E. Flowers, Juliana Gil-Loaiza, Angelika Kübert, Ines Bamberger, Christopher R. Anderton, John Cliff, Sarah Leichty, Roya AminiTabrizi, Jürgen Kreuzwieser, Lingling Shi, Xuejuan Bai, Dusan Velickovic, Michaela A. Dippold, S. Nemiah Ladd, Christiane Werner, Laura K. Meredith & Malak M. Tfaily

Status: published in ***Environmental Science & Technology*** in 2022,

doi: 10.1021/acs.est.1c06772

Contribution (%)	Study design	Data collection	Data analysis	Discussions	Writing
L. Erik Daber	35	35	10	5	5

For a detailed contribution list, see:

https://arizona.figshare.com/articles/dataset/B2WALD_Campaign_Team_and_Contributions/14632662?file=31021090

Study 3

Leaf-level metabolic changes in response to drought affect daytime CO_2 emission and isoprenoid synthesis

S. Nemiah Ladd*, **L. Erik Daber***, Ines Bamberger, Angelika Kübert, Jürgen Kreuzwieser, Gemma Purser, Johannes Ingrisch, Jason Deleeuw, Joost van Haren, Laura K. Meredith & Christiane Werner

*Equal contributions

Status: accepted for publication in ***Tree Physiology*** in 2023,

Manuscript ID: TP-2023-102.R1

Contribution (%)	Study design	Data collection	Data analysis	Discussions	Writing
L. Erik Daber	20	20	50	30	40
S. Nemiah Ladd	25	25	30	30	50
Ines Bamberger	20	10	10	5	0
Angelika Kübert	0	5	5	0	0
Jürgen Kreuzwieser	10	5	5	10	0
Gemma Purser	0	5	0	0	0
Johannes Ingrisch	0	5	0	0	0
Jason Deleeuw	0	10	0	0	0
Joost van Haren	0	5	0	0	0
Laura K. Meredith	5	5	0	5	0
Christiane Werner	20	5	0	20	10

Study 4

Position-specific isotope labelling gives new insights into chiral monoterpene synthesis of Norway spruce (*Picea abies* L.)

L. Erik Daber, Philipp Nolte, Jürgen Kreuzwieser, Mirjam Meischner, Jonathan Williams & Christiane Werner

Status: under major revision in ***Plant Physiology*** in 2023,

Manuscript ID: PP2023-RA-00660D

Contribution (%)	Study design	Data collection	Data analysis	Discussions	Writing
L. Erik Daber	50	40	60	40	70
Philipp Nolte	15	35	25	10	5
Jürgen Kreuzwieser	15	0	5	20	10
Mirjam Meischner	0	25	10	5	0
Jonathan Williams	0	0	0	5	5
Christiane Werner	20	0	0	20	10

Study 5

Chiral monoterpene dynamics of shoots and roots of Norway spruce in response to drought

L. Erik Daber, Jürgen Kreuzwieser, Mirjam Meischner, Jonathan Williams & Christiane Werner

Status: submitted to ***Plant Cell & Environment*** in 2023, Manuscript ID: PCE-23-0691

Contribution (%)	Study design	Data collection	Data analysis	Discussions	Writing
L. Erik Daber	60	80	90	50	75
Jürgen Kreuzwieser	20	10	10	25	10
Mirjam Meischner	0	10	0	5	0
Jonathan Williams	0	0	0	5	5
Christiane Werner	20	0	0	25	10

Appendix: Cumulative studies

Study 1

Ecosystem fluxes during drought and recovery in an experimental forest

Christiane Werner, Laura K. Meredith, S. Nemiah Ladd, Johannes Ingrisch, Angelika Kübert, Joost van Haren, Michael Bahn, Kinzie Bailey, Ines Bamberger, Matthias Beyer, Daniel Blomdahl, Joseph Byron, L. Erik Daber, Jason Deleeuw, Michaela A. Dippold, Jane Fudyma, Juliana Gil-Loaiza, Linnea K. Honeker, Jia Hu, Jianbei Huang, Thomas Klüpfel, Jordan Krechmer, Jürgen Kreuzwieser, Kathrin Kühnhammer, Marco M. Lehmann, Kathiravan Meeran, Pawel K. Misztal, Wei-Ren Ng, Eva Pfannerstill, Giovanni Pugliese, Gemma Purser, Joseph Roscioli, Lingling Shi, Malak M. Tfaily & Jonathan Williams

Status: published in ***Science*** in 2021,

doi: 10.1126/science.abj6789

Study 2

Elucidating Drought-Tolerance Mechanisms in Plant Roots through ^{1}H NMR Metabolomics in Parallel with MALDI-MS, and NanoSIMS Imaging Techniques

Linnea K. Honeker, Gina A. Hildebrand, Jane D. Fudyma, L. Erik Daber, David Hoyt, Sarah E. Flowers, Juliana Gil-Loaiza, Angelika Kübert, Ines Bamberger, Christopher R. Anderton, John Cliff, Sarah Leichty, Roya AminiTabrizi, Jürgen Kreuzwieser, Lingling Shi, Xuejuan Bai, Dusan Velickovic, Michaela A. Dippold, S. Nemiah Ladd, Christiane Werner, Laura K. Meredith & Malak M. Tfaily

Status: published in ***Environmental Science & Technology*** in 2022,
doi: 10.1021/acs.est.1c06772

Study 3

Leaf-level metabolic changes in response to drought affect daytime CO_2 emission and isoprenoid synthesis

S. Nemiah Ladd, L. Erik Daber, Ines Bamberger, Angelika Kübert, Jürgen Kreuzwieser, Gemma Purser, Johannes Ingrisch1, Jason Deleeuw, Joost van Haren, Laura K. Meredith & Christiane Werner

Status: accepted for publication in ***Tree Physiology*** in 2023,

Manuscript ID: TP-2023-102.R1

.

Study 4

Position-specific isotope labelling gives new insights into chiral monoterpene synthesis of Norway spruce (*Picea abies* L.)

L. Erik Daber, Philipp Nolte, Jürgen Kreuzwieser, Mirjam Meischner, Jonathan Williams & Christiane Werner

Status: resubmitted after major revision to ***Plant Physiology*** in 2023,

Manuscript ID: PP2023-RA-00198

Study 5

Chiral monoterpene dynamics of shoots and roots of Norway spruce in response to drought

L. Erik Daber, Jürgen Kreuzwieser, Mirjam Meischner, Jonathan Williams, Christiane Werner

Status: submitted to ***Plant Cell & Environment*** in 2023,
Manuscript ID: PCE-23-0691

Statutory declaration

I, Lars Erik Daber, herewith declare that this thesis comprises only my original work and does not contain any significant amount of unacknowledged work of others. Acknowledgement has been made in the text to all other material used. Where I have consulted the published work of others it is always clearly attributed. Particularly, I did not use help of professional bodies or assistants to complete my work.

This dissertation has not been submitted for the award of any other degree or diploma at any other institution.

I am familiar with the regulations of the examination office at the faculty of Environment and Natural Resources at the University of Freiburg, especially that I am not entitled to use the Dr. rer. nat. title before completion of the doctorate.

Freiburg i.Br., 18.07.2023 _____

Lars Erik Daber

Acknowledgements

Financial support is gratefully acknowledged from the ERC-Project “Biochemical link between plant volatile organic compound (VOC) emissions and CO_2 metabolism - from sub-molecular to ecosystem scales” (ERC consolidator grant 647008 to Christiane Werner) as well as the DFG funded SFB “ECOSENSE - Skalenübergreifende Quantifizierung von Ökosystemprozessen in ihrer räumlich-zeitlichen Dynamik mittels smarter autonomer Sensornetzwerke” (SFB 1537).

Page 71 contains personal information and is therefore not available in the online-version.

Page 72 contains personal information and is therefore not available in the online-version.

Page 73 contains personal information and is therefore not available in the online-version.

Page 74 contains personal information and is therefore not available in the online-version.

Page 75 contains personal information and is therefore not available in the online-version.

Page 76 contains personal information and is therefore not available in the online-version.

Page 77 contains personal information and is therefore not available in the online-version.

Page 78 contains personal information and is therefore not available in the online-version.

Page 79 contains personal information and is therefore not available in the online-version.

Page 80 contains personal information and is therefore not available in the online-version.

Page 81 contains personal information and is therefore not available in the online-version.

Page 82 contains personal information and is therefore not available in the online-version.