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UNIVERSITE DE LIEGE – GEMBLOUX AGRO-BIO TECH

# Exchanges of Biogenic Volatile Organic Compounds Between The Atmosphere and Agricultural Plants/Ecosystems in Controlled and Field Conditions

**Md Ahsan Mozaffar**

Dissertation originale présentée en vue de l'obtention du grade de docteur en sciences agronomiques et ingénierie biologique

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**Summary**

Terrestrial vegetation is a huge source of volatile organic compounds (VOCs) in the Earth's atmosphere. Those biogenic VOCs (BVOCs) are very diverse in composition and many of them react rapidly with the major atmospheric oxidants, thus affecting the oxidation capacity of the atmosphere, air quality and climate. In order to quantify the impact of BVOC fluxes on the atmospheric composition, flux measurements from different plant species were already started a few decades ago. Most of those studies, however, focussed on fluxes from tree species and, despite the fact that 38% of the global land surface area is used for agricultural purposes, the number of BVOC exchange studies on agricultural species is still very limited. Furthermore, agricultural plants/ecosystems are expected to exchange mainly oxygenated VOCs (OVOCs) with the atmosphere and large uncertainties remain about the mechanisms which control the exchanges of those compounds.

This thesis aims at increasing the knowledge on BVOC exchanges between agricultural plants/ecosystems and the atmosphere by focussing on maize, a crop species cultivated on 13% of the global cropland surface area, and on grassland, an ecosystem covering one fourth of the Earth's land surface.

For maize, the main objectives of these investigations were to characterize the type and quantity of BVOCs exchanged and how they vary among the different leaf developmental stages. BVOC fluxes from young up to senescent leaves were investigated in a temperature and light-controlled environmental chamber using dynamic flow-through enclosures and proton transfer reaction mass spectrometry (PTR-MS). BVOC flux intensities as well as their responses to variations in incoming radiation varied strongly among the different leaf developmental stages. Methanol was found to be the highest exchanged compound at all leaf developmental stages. Young leaves exchanged significantly more methanol than leaves at other developmental stages. They showed a complex emission pattern which might be related to the diurnal evolution in leaf growth rate or the diurnal variability in enzyme activity. Particularly, young maize leaves showed strong emission peaks following light/dark transitions, which were impossible to reproduce with state-of-the-art modelling and for which guttation was put forward as a hypothetical emission pathway. Mature leaves showed a less complex response to varying light conditions and emissions could be fairly well reproduced with a dynamic BVOC emission model, by assuming a methanol production function depending both on light and temperature. Finally, both yellow leaves during chlorosis and dry

brown leaves after chlorosis were identified as important methanol sources, with emissions that were no longer correlated with light. Fluxes of other compounds than methanol were followed as well, important ones being acetone/propanal, acetic acid, methyl ethyl ketone/butanal, hexenals and hexenols/hexenyl acetates. Senescent leaves showed a larger diversity of emitted compounds than leaves at the other developmental stages, with strong differences among VOCs in temporal emission profiles. As the first maize leaf from the base of the plant starts senescing long before flowering and this process continues for all the leaves from the base to the top of the plant throughout the growing season, senescence can be an important leaf developmental stage in terms of BVOC emissions from a maize field. Whereas data for BVOC fluxes from senescent maize leaves are not yet available in the literature, fluxes from young up to mature maize leaves were found to be considerably lower than those observed in previous maize studies, except for an ecosystem scale study performed on the same variety.

For grassland ecosystems, the principal objectives of this work were to identify the constitutively exchanged BVOCs and their flux intensity and controlling parameters, and to find out how grazing-induced VOC fluxes differ from the constitutive ones. To meet these objectives, BVOC fluxes were followed simultaneously from side-by-side situated undisturbed and grazed patches in a managed grassland at the Dorinne Terrestrial Observatory (DTO) in the Province of Namur, Belgium, using automated dynamic flow-through enclosures and PTR-MS. For the undisturbed grassland, methanol was the main exchanged compound (emission) among the measured compounds, followed by acetic acid (deposition) and acetaldehyde (deposition). A good positive correlation between fluxes of all the emitted compounds and light intensity, temperature, transpiration and net photosynthesis rates was observed, while deposited compounds showed a relatively good negative correlation with ambient concentration. Strong qualitative and quantitative differences between the BVOC fluxes from the grazed and undisturbed grassland patches were observed, with daytime emissions for all the measured compounds induced by grazing. These induced emissions generally lasted for 2-5 days after the grazing event. Nevertheless, grazing-induced fluxes observed in the current study are typically one to two orders of magnitude lower than those for harvested grassland, where grass is left drying on the field for hay production.

This is the very first investigation on BVOC fluxes from maize at all leaf developmental stages, providing insight on intensity and pattern of BVOC fluxes from this important crop species. Moreover, this is also the first study ever on BVOC fluxes from grassland induced

by grazing, which is a common biotic stress on these ecosystems (60% of the world's agricultural land is grazing land). These findings can be useful to modellers to estimate the total annual BVOC fluxes from these widely distributed ecosystems more correctly. Incorporation of the information in regional and global chemistry and climate models may help to assess the effect of BVOC fluxes on present and future air quality and climate.

**Résumé**

La végétation terrestre est une source massive de composés organiques volatils (COVs) dans l'atmosphère. Ces COV biogéniques (COVBs) ont une composition très diverse et beaucoup d'entre eux réagissent rapidement avec les oxydants atmosphériques majeurs, ayant ainsi un effet important sur la capacité oxydative de l'atmosphère, la qualité de l'air et le climat. Afin de quantifier l'impact des émissions de COVB sur la composition atmosphérique, des mesures d'émissions de différentes plantes ont débutés il y a déjà quelques décades. Par contre, la plupart de ces études se sont focalisées sur les émissions foliaires d'arbres et, bien que 38% de la surface terrestre globale est dédiée à l'agriculture, le nombre d'études sur les échanges de COVB par les espèces agricoles est encore très limité. De plus, les plantes/écosystèmes agricoles sont supposés échanger surtout des COV oxygénés (COVOs) avec l'atmosphère et il reste de grandes incertitudes sur les mécanismes qui contrôlent les échanges de ces composés.

Cette thèse a pour objectif d'étendre les connaissances sur les échanges de COVB entre les plantes/écosystèmes agricoles et l'atmosphère en focalisant sur le maïs, qui est une espèce occupant 13% de la surface agricole mondiale, et sur une prairie, un écosystème couvrant un quart des surfaces émergées.

Concernant le maïs, les objectifs principaux étaient d'identifier les COVBs échangés, de quantifier leurs flux et d'étudier la variation des flux en fonction des stades de développement des feuilles. Les flux ont été mesurés dans une chambre environnementale en utilisant des cuvettes dynamiques et la technique de spectrométrie de masse par transfert de protons (PTR-MS). L'intensité des flux de COVB ainsi que la réponse des flux à la variation de la radiation photosynthétiquement active variaient fortement entre les différents stades de développement de la feuille. Il s'est avéré que le méthanol était le composé avec le plus grand taux d'échange pour tous les stades de développement. Les feuilles jeunes échangeaient beaucoup plus de méthanol que les feuilles ad' autres stades. En plus, la variation diurne des flux était très complexe et les facteurs contrôlant les émissions ne sont toujours pas bien compris. En particulier, les émissions de méthanol par les jeunes feuilles de maïs montraient des pics transitoires après la transition jour/nuit qui n'ont pas pu être reproduits par les modèles d'émissions les plus récents et pour lesquels la guttation a été avancée comme une voie d'émission potentielle. La réponse des émissions de méthanol par les feuilles adultes à la variation de la lumière s'est révélée moins complexe et a pu assez bien être reproduite par un

modèle dynamique d'émission de COVB en tenant compte d'une production de méthanol qui dépend à la fois de la température et de la lumière. Enfin, les feuilles jaunâtres pendant la chlorose et les feuilles brunes et sèches après la chlorose ont été identifiées comme des sources importantes de méthanol et ces émissions n'étaient plus corrélées à la lumière. Les flux d'autres composés que le méthanol ont également été mesurés, les plus importants étant ceux d'acétone/propanal, d'acide acétique, de methyl éthyl cétone/butanal, d'hexénals et d'hexénols/hexényls acétates. La diversité des composés émis par les feuilles sénescentes était plus grande que dans les autres stades de développement. De grandes différences dans les profils temporels des émissions ont été observées parmi les COVBs provenant des feuilles sénescentes.

Comme la feuille de maïs à la base de la plante entre en senescence bien avant la floraison et que ce processus se poursuit pour toutes les feuilles de la base jusqu'au sommet de la plante au long de la saison de croissance, la senescence peut être un stade de développement important en terme d'émission de COVB pour un champ de maïs. Alors que des taux d'émission de COVB par les feuilles sénescentes de maïs ne sont pas encore disponibles dans la littérature, ceux que nous avons obtenus pour les feuilles jeunes et adultes sont nettement inférieurs par rapport aux autres études publiées, à l'exception d'une étude à l'échelle d'un écosystème sur la même variété de maïs.

Concernant la prairie, les objectifs principaux de cette recherche étaient d'identifier les COVB constitutifs échangés avec l'atmosphère et de déterminer les intensités des flux et les facteurs qui régulent les émissions, ainsi que d'étudier les émissions de COVB induites par le pâturage. Pour atteindre ces objectifs, les flux de COVB émis par des carrés de prairie intacts et pâturés, situés côte à côte dans une prairie de l'Observatoire Terrestre de Dorinne (OTD) en Province de Namur, Belgique, ont été mesurés simultanément en utilisant des cuvettes dynamiques et le PTR-MS. Le méthanol était le composé le plus échangé (émission) parmi les COVB mesurés, suivi par l'acide acétique (déposition) et l'acétaldéhyde (déposition). De bonnes corrélations positives entre les flux de tous les composés émis d'une part et l'intensité lumineuse, la température de l'air et les taux de transpiration et de photosynthèse nette d'autre part ont été observées. Par contre, les flux des COVB déposés étaient assez bien négativement corrélés avec la concentration ambiante. De grandes différences qualitatives et quantitatives ont été constatées entre les flux de COVB des carrés de prairie intacts et pâturés, avec des émissions de jour pour tous les composés, induites par le pâturage. Ces émissions induites duraient en général jusqu'à 2-5 jours après le pâturage mais elles étaient typiquement

un à deux ordres de grandeur plus petites que celles observées pour les prairies fauchées, lorsque l'herbe sèche sur place pour la production de foin.

Les recherches effectuées au cours de cette thèse constituent les toutes premières mesures de flux de COVB sur tous les stades de développement des feuilles de maïs, et elles ont résulté en une meilleure connaissance de l'intensité et de la diversité des flux de COVB de cette espèce agricole importante. De plus, ceci est la première étude sur les flux de COVB induits par le pâturage sur une prairie, le pâturage étant un facteur de stress biotique commun dans ces écosystèmes (60% de la surface agricole globale est pâturée). Les résultats peuvent être utiles aux modélisateurs afin de mieux estimer le flux global annuel de ces écosystèmes largement répandus. L'incorporation de ces informations dans les modèles régionaux et globaux de chimie atmosphérique et du climat peut aider à évaluer l'effet des flux de COVB sur la qualité de l'air et le climat actuels et futurs.

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# Chapter 1: Introduction

## 1.1 Volatile Organic Compounds (VOCs) in the atmosphere

In the field of atmospheric chemistry volatile organic compounds (VOCs) are generally considered as gaseous organic trace species other than carbon dioxide ($CO_2$) and carbon monoxide (CO) (Kesselmeier and Staudt, 1999). Although methane ($CH_4$) is the most abundant gaseous hydrocarbon in the atmosphere, it is often excluded from the VOC group due to its low reactivity (atmospheric lifetime of ~10 years) and its importance as a greenhouse gas (IPCC, 2014). Consequently atmospheric scientists often use the acronym NMVOCs (non-methane VOCs) when referring to atmospheric VOCs. Other acronyms have been used as well depending on the origin, chemical composition and properties of the VOCs, like AVOCs (anthropogenic VOCs), BVOCs (biogenic VOCs), OVOCs (oxygenated VOCs), and ORVOCs (other reactive VOCs) (Kesselmeier and Staudt, 1999). According to the Environmental Protection Agency (EPA) in the United States, VOCs are considered to be those organic compounds having a vapour pressure greater than 10 Pa at 25 °C, a boiling point up to 260 °C at atmospheric pressure and 15 or less carbon atoms (Williams and Koppmann, 2007). Compounds with many different functional groups (e.g. alkanes, alkenes, alcohols, aldehydes, ketones, carboxylic acids) are included in the VOC family. Among them, isoprenoids like isoprene and monoterpenes are the most prominent compounds followed by alcohols and carbonyls. Atmospheric concentrations of individual biogenic NMVOCs range between a few pptv and several ppbv (Kesselmeier and Staudt, 1999). Despite their low concentrations, VOCs have profound effects on the atmosphere (Atkinson, 2000). They are the "fuel" which keeps oxidative atmospheric photochemistry running (Williams and Koppmann, 2007). Hence, their sources, sinks and effects on atmospheric chemistry and composition are the subject of much current research (Williams and Koppmann, 2007).

## 1.2 Sources of atmospheric VOCs

### 1.2.1 Anthropogenic VOC sources

The global anthropogenic VOC emissions are estimated to be 110 Tg C $year^{-1}$ (Piccot et al., 1999). Almost everything we do in daily life e.g. driving a car (Ho et al., 2009), painting a house (Fortmann et al., 1998), cooking (McDonald et al., 2003), making a fire (Schauer et al., 2001), results in the release of VOCs. Therefore, sources of anthropogenic VOCs are widely

distributed, and the major sources include production, treatment, storage and distribution of fossil fuels, combustion processes, the use of volatile solvents and solvent-containing products, industrial production processes, and waste management. The most common VOCs emitted by human activities are ethane, propane, butanes, pentanes, hexanes, heptanes, octanes, cycloparaffins, ethene, propene, ethylene, benzene, toluene, xylene, formaldehyde, acetaldehyde, and isopropanol (Friedrich and Obermeier, 1999).

### 1.2.2 Biogenic VOC sources

Total biogenic NMVOC (i.e. BVOC) emissions are considered to be approximately ten times larger than total anthropogenic emissions (Guenther, 2002), and are estimated to be around 1000 Tg C $year^{-1}$ (Guenther et al., 2012). Terrestrial vegetation is the major source of BVOCs. More than 1700 different BVOCs have been identified from 90 different plant families belonging to both angio- and gymnosperms (Knudsen et al., 2006). BVOCs include terpenoids (isoprene, monoterpenes and sesquiterpenes) as well as different kinds of alkanes, alkenes, alcohols, acids, carbonyls, esters, and ethers (Kesselmeier and Staudt, 1999). The estimated annual global emissions of the major BVOCs or families of BVOCs with their corresponding sources are shown in Table 1.1 below. Different plant families emit different subsets of VOCs, which are produced in many different plant tissues and compartments and are the products of diverse physiological processes (Fall, 1999). This is illustrated in Figure 1.1 by a hypothetical "VOC tree" which emits all the major plant-derived VOCs plus floral scent VOCs. The single most important BVOC in the earth system is isoprene (Laothawornkitkul et al., 2009) which comprises about half of the total global BVOC emission estimated using MEGAN2.1 (Guenther et al., 2012). α-pinene, β-pinene, t-β-ocimene, limonene, methanol, ethanol, acetaldehyde, acetone, ethane, and propene together contribute another 30% of total BVOC emissions estimated using the abovementioned model. As a result of their predominance and their high atmospheric reactivity the most studied BVOCs are therefore the isoprenoid compounds, which are mainly emitted by forest ecosystems. Consequently many studies have been carried out in the past on BVOC emissions from trees and shrubs, and much less from agricultural plants, which are the subject of this thesis. According to a recent European plant-specific BVOC emission inventory (Karl et al., 2009), 25% of the pan-European BVOC emissions come from agricultural land use. More details about BVOC fluxes from agricultural ecosystem can be found in Section 1.8. Terpenoids (isoprene + monoterpenes + sesquiterpenes) contribute only 39% to the total BVOC emission from agriculture, in contrast to 61% for oxygenated VOCs

(OVOCs) (Karl et al., 2009). OVOCs started to receive more attention in the past one and a half decade as the technology to measure their emissions has greatly improved (Steiner and Goldstein, 2007).

Besides terrestrial vegetation, soil microorganisms, plant roots and litter also act as sources or sinks of VOCs (Insam et al., 2010; Penuelas et al., 2014 and references therein). Compared to the aboveground vegetation, however, BVOC emissions from soil were reported to be 1-2 orders of magnitude lower, and dependent on ecosystem and environmental conditions (Penuelas et al., 2014 and references therein).

Table 1.1: Estimated annual global emission and atmospheric lifetime of the major BVOCs or families of BVOCs with their sources (data are derived from Fall (1999) and references therein)

| Species | Primary natural sources | Estimated annual global emission (Tg C) | Atmospheric lifetime (days) |
|---|---|---|---|
| Methane* | Wetland, rice paddies | 319-412 | 4000 |
| Isoprene | Plants | 175-503 | 0.2 |
| Monoterpenes | Plants | 127-480 | 0.1-0.2 |
| Dimethylsulfide | Marine phytoplankton | 15-30 | <0.9 |
| Ethylene | Plants, soil, oceans | 8-25 | 1.9 |
| Other reactive VOCs (e.g. acetaldehyde, 2-methyl-3-buten-2-ol, hexenal family) | Plants, oceans | ~260 | <1 |
| Other less reactive VOCs (e.g. methanol, ethanol, formic acid, acetic acid, acetone) | Plants, soil, oceans | ~260 | >1 |

**For the completeness of information, data related to methane are also presented in this table*

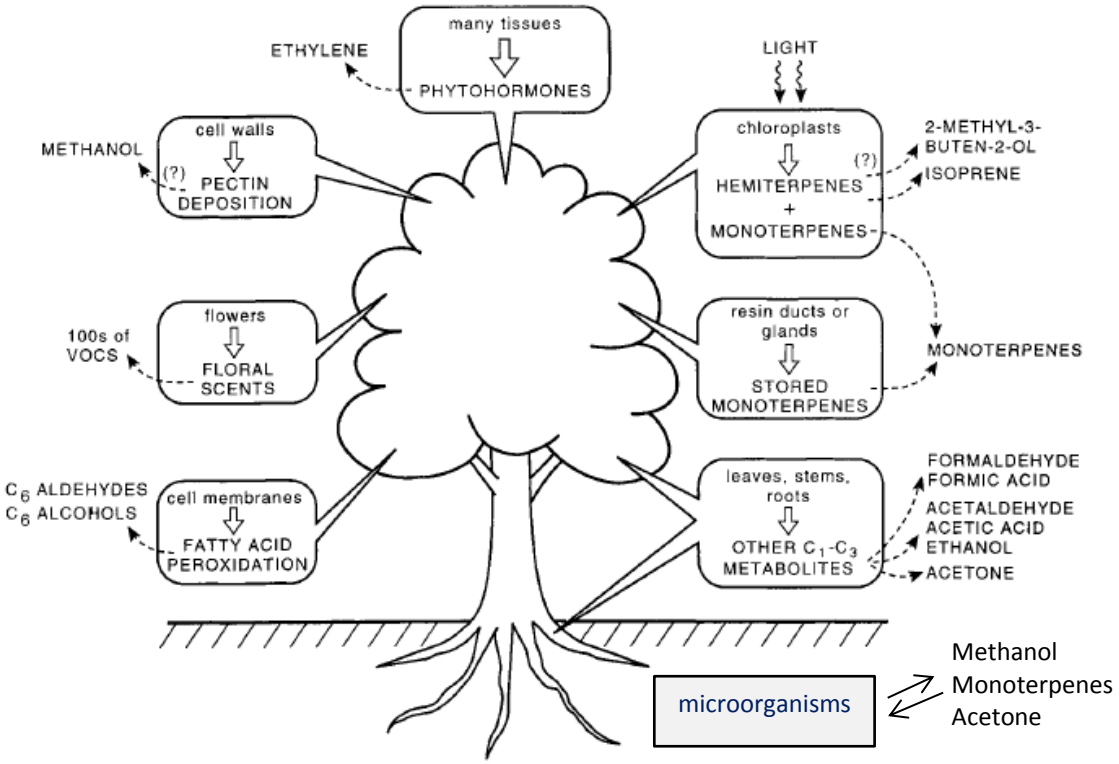



Figure 1.1: A hypothetical "VOC tree" illustrates that plants have the metabolic potential to produce and emit a variety of VOCs. The potential plant tissues and compartments for VOC formation are indicated, as well as VOC exchanges between soil microorganisms and the atmosphere (this figure is adapted from Fall, 1999).

## 1.3 Production mechanisms, emission drivers, and ecophysiological role of some common BVOCs

In the following subsections production mechanisms, emission drivers or controls over emissions and the eco-physiological role of some of these common BVOCs will be briefly discussed. As this dissertation deals with BVOC fluxes from agricultural crops, which mainly emit OVOCs, the focus of those subsections will mainly be on OVOCs. For the sake of completeness, however, isoprenoids will be briefly considered as well.

### 1.3.1 Methanol

Pectin demethylation in plant cell walls is the major source of methanol in the atmosphere (Galbally and Kristine, 2002). This process is linked to any change in cell wall structure like expansion during growth or loosening during fruit ripening, senescence and abscission (Willats et al., 2001). Methanol is a by-product of this process and escapes to the atmosphere

only through the stomata (unless the plant is injured).Factors which influence cell wall structure changes control methanol production rates.

Abundant amounts of polysaccharide pectin are present in the primary cell walls of most land plants (Willats et al., 2001). During cell wall formation and expansion, highly methylated pectins are secreted into the cell wall. Cellulose and hemicellulose polymers, which are assembled in the cell wall, are held together by noncovalent associations. One of these associations results from the demethylation of pectin through the action of the enzyme pectin methyl esterase (PME) which ultimately produces calcium-pectate gels that are thought to rigidify and strengthen the cell wall. Methanol is produced as a by-product in this process (Fall, 2003). More details about the methanol production can be found in Fall, 2003. Because of the link between plant growth and methanol emissions, leaf age influences methanol emissions, young leaves emitting more methanol than mature leaves (Huve, 2007; Aalto et al., 2014). The pectin demethylation process also occurs during aging and senescence of plant tissues (Nemecek-Marshall et al., 1995), resulting in high methanol emissions at those conditions as well.

Phenological processes of the vegetation control methanol production (Bracho-Nunez et al., 2011). High temperature exponentially increases methanol emission rates probably due to increasing rates of enzymatic reaction of production (Harley et al., 2007; Folkers et al., 2008). Wounding and herbivore damage also induce methanol emission from plants (Hann et al., 2014; Loreto et al., 2006) by promoting the activation of PME (Komarova et al., 2014 and references therein).

Stomatal conductance controls methanol emission rates because of the high solubility of methanol in water (MacDonald and Fall, 1993; Nemecek-Marshall et al., 1995; Niinemets and Reichstein, 2003a). One consequence of this is that drought stress reduces methanol emission rates by reducing stomatal conductance (Bourtsoukidis et al., 2014). Covariation of methanol emissions with light has also been observed (Harley et al., 2007; Koppmann and Wildt, 2007), and the effect of light on stomatal conductance has been put forward as a potential reason (Harley et al., 2007). The state-of-the-art of drivers of methanol emissions is described in detail in chapter 3, Sect. 3.1.

The role of methanol in plant defence is poorly understood. It can be perceived by the intact leaves of the damaged plant and also the neighboring plants as a sign of damage/pathogen attack and induce a defence response (Hann et al., 2014; Komarova et al.,

2014 and references therein). Enhanced methanol production in plants was reported to protect plants from insects (Dixit et al., 2013).

### 1.3.2 Acetone

Two well-characterized biochemical processes or mechanisms for biogenic acetone production have been reported hitherto: (a) cyanogenic formation of acetone in plants and (b) enzymatic and non-enzymatic acetoacetate decarboxylation in bacteria, animals and humans. In the following paragraphs, a brief description of the two biosynthesis processes and associated emission drivers will be given.

Cyanogenic plants synthesize acetone and hydrogen cyanide after wounding to deter herbivores. Details about the cyanogenic formation of acetone are given in Fall, 2003. To the best of our knowledge, the production mechanism of acetone in undisturbed plants has not been elucidated yet. Acetone is produced in bacteria and humans by decarboxylation of acetoacetic acid or its ionized form, acetoacetate. In bacteria the reaction is enzymatic, and in humans and other animals it is non-enzymatic. More details about these processes can be found in Fall, 2003.

Acetone emission rates were reported to increase exponentially with temperature (Filella et al., 2007). The reason for the increase of acetone emission with temperature is not clearly understood (Filella et al., 2007). One reason could be an increase of enzymatic biosynthesis with temperature. Studies have suggested that high ozone concentration in the air results in increased acetone emission rates by increasing production rates as well (Cojocariu et al., 2005).

Acetone emission rates were also found to increase with increasing light intensity at constant leaf temperature (Min and Wildt, 2002). As acetone dissolves in the transpiration stream, increased stomatal conductance under increasing light intensity may enhance acetone emissions. On the other hand, acetone emissions were noticed to decrease with soil water availability due to decrease in stomatal conductance (Filella et al., 2009). Effect of elevated $CO_2$ concentration on acetone emission is inconclusive. Acetone emissions from *Quercus ilex* were found to increase under elevated $CO_2$ concentration in autumn but decreased in summer. However, opposite results were observed for *Quercus pubescens* (Kreuzwieser et al., 2002).

### 1.3.3 Acetaldehyde

So far three metabolic pathways for acetaldehyde synthesis in plants are known: (a) ethanolic fermentation in anaerobic roots (Kreuzwieser et a., 1999), (b) the pyruvate overflow mechanism (Karl et al., 2002), and (c) fatty acid peroxidation by reactive oxygenated species (ROS) (Jardine et al., 2009). A brief description of these three biosynthesis mechanisms with emission drivers and the ecophysiological role of acetaldehyde emission will be given below.

In the ethanolic fermentation pathway, glucose is converted to pyruvate in the roots of flooded plants. Pyruvate is subsequently converted to acetaldehyde, which in turn is reduced to ethanol. The ethanol produced in the roots is carried to the leaves by xylem sap, where it is oxidized to acetaldehyde (Fall, 2003 and references therein).

During a light/dark transition, cytosolic pyruvic acid levels in leaves increase rapidly. Enzyme (pyruvate decarboxylase) in the cytosol acts as a sensor, converts excess pyruvate to acetaldehyde and reduces cytosolic acidification (Harry & Kimmerer 1991). The produced acetaldehyde can either be oxidized to acetate or be partitioned in the leaf air space and released to the atmosphere (Fall, 2003 and references therein).

It is well known that plants produce ROS in response to stresses like wounding, ozone damage, pathogen attack (Kotchoni and Gachomo, 2006; Cojocariu et al., 2005; Langebartels et al., 2002). Acetaldehyde can also be produced as an intermediate metabolite of fatty acid peroxidation by ROS generated by stresses (Jardine et al., 2009).

Enhanced acetaldehyde emissions are also noticed under high temperature stress (Loreto et al., 2006) probably due to increase in enzymatic biosynthesis rates. Different kinds of biotic and abiotic stresses give rise to acetaldehyde emissions (Kotchoni and Gachomo, 2006; Cojocariu et al., 2005; Langebartels et al., 2002) by increasing its production rate. Flooding or anaerobic conditions of plants roots increase acetaldehyde production rates as well (Rottenberger et al., 2008).

Acetaldehyde being easily dissolved in leaf water, its emission is also observed to be controlled by stomatal conductance (Rottenberger, et al., 2008), as is the case for methanol and acetone. The effect of elevated $CO_2$ on acetaldehyde emission is not conclusive yet. Acetaldehyde emissions were observed to increase under elevated $CO_2$ concentration in autumn but slightly decreased in summer for *Quercus ilex* (Kreuzwieser et al., 2002).

Acetaldehyde emission from damaged tissue may help to prevent infection because of its antibiotic properties (Utama et al. 2002). As its emission is observed under stress conditions, it may play an important role in plant defence (Jardine et al., 2009) and it may activate the expression of plant defence genes (Tadege et al., 1998).

### 1.3.4 Acetic acid

Little information about acetic acid biosynthesis is available in the literature. It could be produced in plants from Acetyl coenzyme A (acetyl-CoA). Under metabolic conditions where high levels of acetyl-CoA are generated, such as during fatty acid oxidation, some hydrolysis of acetyl-CoA might occur, leading to the formation of acetic acid (Fall, 1999). Besides, ethanol produced in flooding-stressed plant roots (see §1.3.3) gets oxidized in leaves to produce acetic acid (Kreuzwieser and Rennenberg, 2013).

Cell growth and expansion could control acetic acid production in plants as higher emissions were observed from young foliage than from mature leaves (Staudt et al., 2000). Flooding stress on plants could increase acetic acid biosynthesis as high emissions were noticed from flooding-stressed plants (Rottenberger, et al., 2008).

Stomatal conductance was found to control acetic acid emission from leaves (Gabriel et al., 1999; Rottenberger, et al., 2008) due to its high water solubility. A positive correlation between acetic acid emissions and light and temperature has been observed (Staudt et al., 2000). Concentration of acetic acid in the ambient air also controls exchanges of acetic acid between plants and the atmosphere (Staudt et al., 2000).

### 1.3.5 Green leaf volatiles

Under stress conditions like herbivore attack or pest infestation, pathogen attack and mechanical damage, plants produce/emit green leaf volatiles (GLVs). GLVs consist of a family of C6 aldehydes, alcohols and esters e.g. hexenals, hexenols, hexenyl acetate. GLVs are produced from fatty acid in chloroplast. Physical damage or stress to cells triggers the release of poly-unsaturated C18 fatty acids from cellular membranes which are enzymatically converted to C6 aldehydes in the LOX pathway (Scala et al., 2013; Dudareva et al., 2013). The C6 aldehydes are then reduced to C6 alcohols which are eventually transformed into esters. Whereas alpha-linolenic acid is the precursor for unsaturated C6 compounds, linoleic acid gives rise to the saturated C6 compounds (Scala et al., 2013). The GLV biosynthesis procedure is elaborately described elsewhere (Scala et al., 2013; Dudareva et al., 2013).

Small quantities of GLVs are emitted constitutively from plant tissue but they are quickly released in large amounts after stress (D'Auria et al., 2007). Stresses like mechanical wounding (Fall et al., 1999), herbivore damage (Turlings and Loughrin, 1995), high temperature (Jardine et al., 2015), high light intensity (Loreto et al., 2006), light to dark transition (Graus et al., 2004), and drought (Jardine et al., 2015) positively influence GLVs production rates.

Z-3-hexenal and E-2-hexenal act as an antimicrobial agent by inhibiting bacteria and pathogenic fungus growth at the wounded site (Kishimoto et al., 2008; Prost et al., 2005). A role for plant-plant communication and priming defence against insect attack has also been reported for Z-3-hexenal (Engelberth et al., 2004). GLV emission is also considered as an indirect defence mechanism for plants to attract predators and parasitoids of herbivore insects (Mumm et al., 2010). Moreover, GLVs have a repellent effect on herbivores as well (Hildebrand et al., 1993).

### 1.3.6 Isoprene

Isoprene (2-methyl-1,3-butadiene) is synthesized in chloroplasts from two five-carbon precursors, dimethylallyl diphosphate (DMAPP) and its isomer isopentenyl diphosphate (IPP) (Li and Sharkey, 2013) by isoprene synthase enzyme (Silver and Fall, 1995). A detailed description of isoprene synthesis in plants can be found in Dudareva et al. (2013) and Maffei (2010).

Isoprene emissions mainly occur through the stomatal pores, and emission through the cuticle is expected to be negligible (Fall and Monson, 1992). Its emissions are characterized by strong temperature and light dependencies (Alves et al., 2014). Isoprene emission rates increase with increasing temperature up to 40-45 °C due to increase of isoprene synthesis rates. Above that temperature emission rates decrease due to isoprene synthase enzyme inactivation (Sharkey, 2005; Li et al, 2011, Niinemets and Sun, 2014). The light dependence of isoprene emission can be explained by the close coupling between isoprene synthesis and photosynthesis via ATP requirement and carbon substrate availability (Harley et al., 1994). Moreover, it was observed that isoprene emission rates decrease with increasing $O_3$ concentration due to a decrease of isoprene synthase gene expression (Calfapietra et al., 2007). The effect of elevated $CO_2$ concentration on isoprene emission is unclear: both increasing and decreasing emission rates have been observed (Sun et al., 2013; Scholefield et al., 2004). The response of elevated $CO_2$ concentration on isoprene emission is species

dependent, a comprehensive review about it can be found in Calfapietra et al. (2013). The effect of drought stress on isoprene emissions depends on the level of stress: mild stress can lead to increased emission (Genard-Zilinski et al., 2014; Niinemets et al., 2010), whereas emission rate decreases under high drought stress conditions (Sharkey and Loreto et al., 1993; Fang et al., 1996).

Studies suggest that isoprene has a thermotolerance property, it protects the photosynthetic apparatus from high temperature (Behnke et al., 2007; Sharkey et al., 2007; Velikova et al., 2012). Isoprene also has antioxidant properties, it minimizes oxidative stresses induced by ozone, drought, salinity (Schnitzler et al., 2010 and references therein).

### 1.3.7 Monoterpenes

Monoterpenes have two isoprene units which are also produced in the chloroplast with the help of terpene synthases (Maffei 2010; Dudareva et al., 2013). Geranyl diphosphate is the precursor of monoterpenes which are produced from dimethylallyl diphosphate and isopentenyl diphosphate (Wise and Croteau, 1999; Dudareva et al., 2013). Monoterpenes production in plants is explained in detail by Dudareva et al. (2013).

Monoterpenes can be released through the stomata as soon as they are produced (Li and Sharkey, 2013). Monoterpenes can also be stored in structures such as resin ducts, trichomes and glands in leaf tissue, and emitted later by diffusion through the cuticular layer and stomata. Monoterpenes emission from the storage pools increases exponentially with increasing temperature (Guenther et al., 1993; Grote et al., 2013). On the other hand, *de novo* synthesized monoterpenes emissions have a temperature optimum at 37-40 °C (Copolovici et al., 2005). Monoterpenes that are emitted directly after production showed light dependent emission (Dindorf et al., 2006; Demarcke et al., 2010; Nishimura et al., 2015). Similar to isoprene, studies on the effect of elevated $CO_2$ on monoterpene emission have shown widely varying results: increase, decrease or no change of emissions, all possible results are observed (Staudt et al., 2000; Loreto et al., 2001; Constable et al., 1999; Calfapietra et al., 2013). Monoterpenes emission was observed to increase under elevated $O_3$ concentration as oxidative stress was stimulating monoterpenes production (Loreto et al., 2004). Some studies have reported enhanced emission of monoterpenes under drought stress (Blanch et al., 2009; Wu et al., 2015) and some have reported the opposite (Lavoir et al., 2009; Plaza et al., 2005; Simpraga et al., 2011). The effect of drought stress on monoterpenes emissions depends on

the level of stress: while severe stress reduces emissions, moderate stress has no effect on emissions (Possell and Loreto, 2013).

Some studies suggest that monoterpenes act as a defence compound against pathogens and herbivores (Cates, 1996; Xu et al., 2016). They also help reproduction by attracting pollinators (Pichersky and Gershenzon, 2002).

## 1.4 Fate of VOCs in the atmosphere

After being emitted into the atmosphere, VOCs undergo one or more removal (sink) processes like photochemical oxidation, nucleation, photolysis, and deposition (Fig 1.2). These removal processes determine their lifetime in the atmosphere shown in table 1.1.

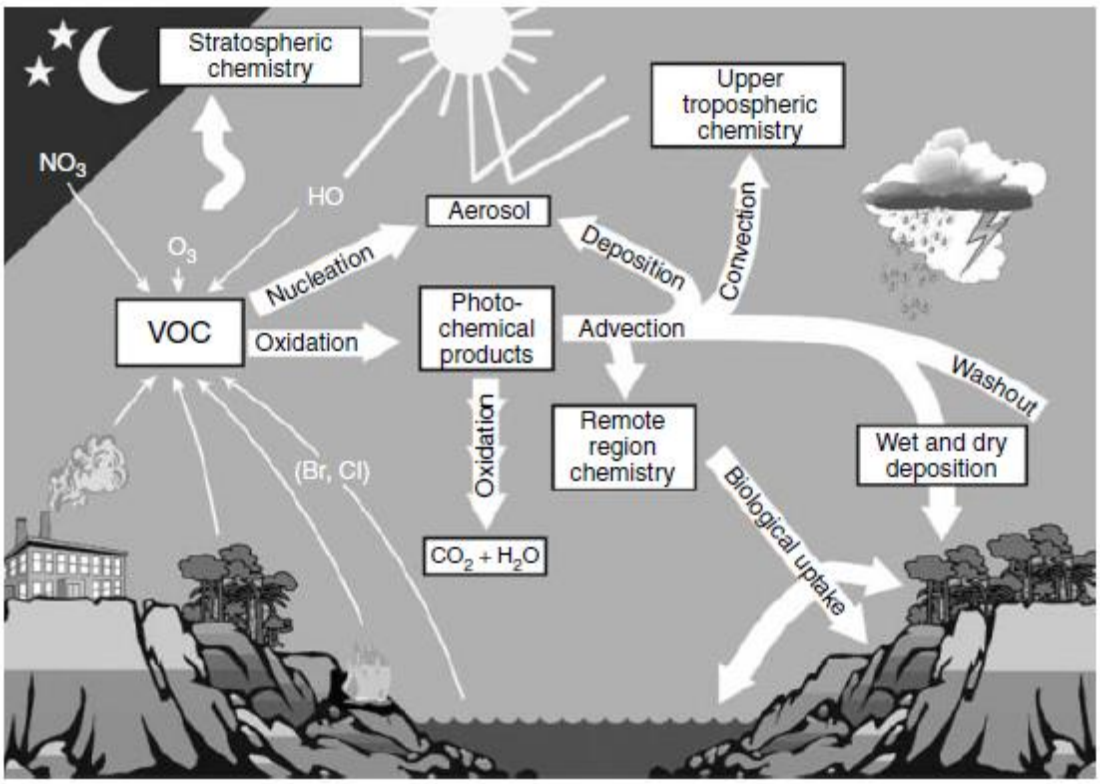
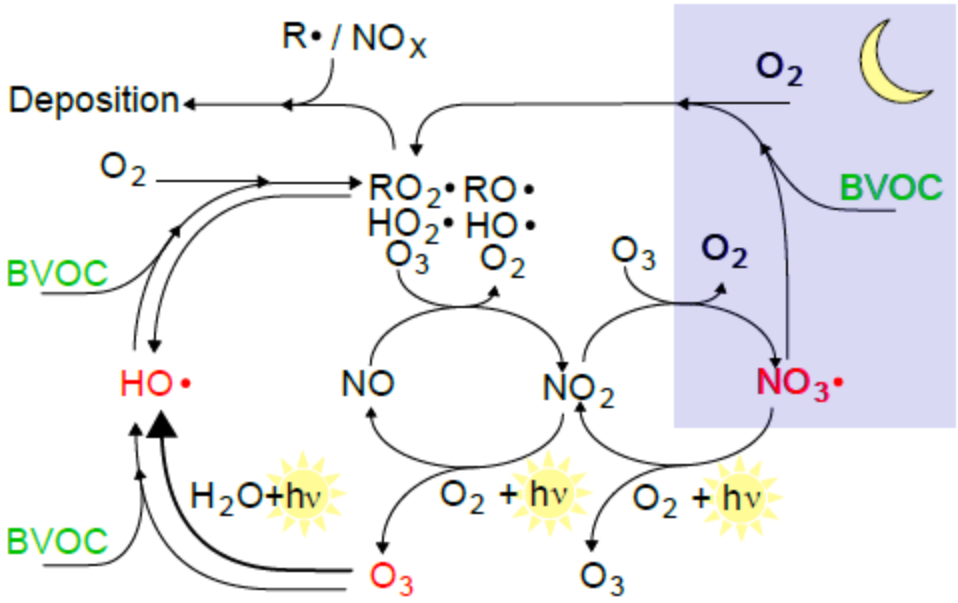



Figure 1.2: Schematic representation of the various processes which determine the fate of VOCs in the atmosphere (this figure is collected from Williams and Koppmann, 2007).

### 1.4.1 Gas phase oxidation

The most important sink for VOCs in the atmosphere is chemical oxidation in the gas phase by $OH^{\bullet}$, $O_3$ and $NO_3^{\bullet}$ (and to a lesser extent by halogen radicals) (Atkinson 1994; Jenkin et al., 2003; Saunders et al., 2003), with direct consequences on the oxidation capacity of the atmosphere (Hewitt et al., 1999). Among these oxidants, $OH^{\bullet}$ plays a predominant role in day-time VOC oxidation. It is mainly produced by photolysis of tropospheric $O_3$ and the subsequent reaction of the resulting oxygen atom with water vapour (Fig. 1.3). In addition, ozonolysis of alkenes is also a small source of OH radicals. At night, VOC oxidation is mainly driven by $NO_3^{\bullet}$ which are mainly generated by the reaction of $NO_2$ with $O_3$ (Monks et al. 2005). The reactions of $OH^{\bullet}$ and $NO_3^{\bullet}$ with VOCs produce alkyl and substituted alkyl radicals, respectively, which subsequently react with $O_2$ to generate organic peroxy radicals. The reaction of these peroxy radicals with NO (in $NO_x$-rich environments) results in the formation of alkoxy radicals and $NO_2$. Photodissociation of $NO_2$, in turn, results in atomic oxygen radicals which readily combine with molecular oxygen to form $O_3$ (Atkinson, 2000 Isaksen et al., 2009; Atkinson and Arey, 2003; Jacob, 1999). Besides being harmful to humans and plants, tropospheric ozone is a potent greenhouse gas which is responsible for global warming (IPCC, 2014). Oxides of nitrogen are emitted into the troposphere from soils, natural fires, combustion processes, and formed in situ in the troposphere from lightning (Atkinson, 2000; and references therein). The alkoxy radicals formed during VOC oxidation undergo complex chemical reactions leading to the formation of different kinds of low vapour pressure organic compounds (alcohols, carbonyls, acids) which may partition between the gas and the particle phase and therefore contribute to secondary organic aerosol (SOA) (Hoffmann et al., 1997).




Figgure 1.3: Schematic diagram of VOC oxidation in the atmosphere (this figure is collected from Demarcke, 2011).

### 1.4.2 Other VOC loss processes

VOCs which are only moderately reactive with the main atmospheric oxidants (e.g. acetone) can be transported to the upper troposphere where they can absorb sufficiently energetic solar radiation and dissociate to smaller fragments (Atkinson, 2000). Dry deposition to surfaces such as vegetation (Doskey et al., 2004) or aerosols (Cousins and Mackay, 2001) is another efficient way for the removal of atmospheric VOCs. Some VOCs are also removed by wet deposition in rain (Fornaro and Gutz 2003). Direct uptake by plants can also be an effective removal process for some VOCs (Kuhn et al., 2002; Seco et al., 2007; Laffineur et al., 2012; Wohlfahrt et al., 2015). The rate of uptake by plants through the stomata is dependent on the stomatal conductance and the VOC concentration differences between the ambient air and the substomatal cavities of the leaves.

## 1.5 Link between BVOC emissions and climate and potential impact of climate change on the emissions

BVOC emissions not only affect air quality by generating tropospheric ozone and fine particulate matter, they also exert an indirect influence on climate. Indeed, tropospheric ozone is also a well-known greenhouse gas, accounting for about 15% of the net radiative forcing attributed to human activities since the beginning of the industrial era (IPCC, 2014). Moreover, as mentioned in the previous paragraph, VOC oxidation products contribute to

SOA, which also exerts an important influence on the radiation balance of the earth by reducing the incoming solar radiation through scattering and reflection. Moreover, SOA is a major source of cloud condensation nuclei (CCN) (Merikanto et al., 2009) which are required for cloud formation, and clouds absorb and reflect the long-wave terrestrial radiation back to the earth surface. A large uncertainty is associated with the production rate of SOA due to limited knowledge in aerosol formation processes and to the uncertainty related to SOA precursors. All this results in large uncertainties on the contributions of aerosols to radiative forcing (IPCC, 2014).

The main sink of methane, another potent greenhouse gas, in the troposphere is reaction with OH radicals. As the reaction with VOCs is also an important sink of OH radicals, atmospheric VOCs control to some extent the sink strength and therefore the lifetime of methane in the atmosphere. Long term changes in VOC concentrations may thus indirectly increase the concentration of methane in the troposphere (Kaplan et al., 2006).

Global warming, drought, increased $CO_2$ and $O_3$ concentrations, and increase in UVB radiation are all effects of climate change which may influence future BVOC emissions. Numerous studies have been dedicated to the impact of climate change on BVOC emissions, nevertheless, there is still a lack of precise answer to the question of how the future BVOC emissions will be under all these global change components (Penuelas and Staudt, 2010; and references therein).

The increase in global mean surface temperature by the end of the 21$^{st}$ century relative to 1850-1900 is projected to likely exceed 1.5 °C (IPCC, 2014, for RCP4.5, RCP6.0 and RCP8.5 scenarios). Warming has numerous direct and indirect effects on BVOC fluxes. It is known that the emissions of most BVOCs exponentially increase with temperature (Filella et al, 2007) because of an increased activity of the enzymes involved in BVOC synthesis. As global temperature rises, BVOC emissions are thus expected to increase sharply unless plants adapt to the high temperature conditions. An indirect effect of global warming is the lengthening of the growing season. Longer growing seasons could lead to more litter fall on the ground (Cornelissen et al., 2007), which brings extra nutrients to the soil and could increase BVOC emissions (Harley et al., 1994).

According to climate models, precipitation will reduce in the Mediterranean type climates and semiarid regions, which will enhance drought and associated heat stress. On the other hand, precipitation will increase at the higher latitudes (IPCC, 2014). These changes in water

(resource) availability could change BVOC fluxes. On the one hand, drought stress might decrease BVOC emission (Bourtsoukidis et al., 2014) by affecting stomatal conductance and producing diffusive and biochemical limitation to photosynthesis (Flexas et al., 2006). On the other hand, high soil water availability might increase BVOC emissions (Bourtsoukidis et al., 2014). However, the effect of drought on BVOC fluxes depends on the level of stress or damage caused to the plant. Severe drought might decrease BVOC emissions (Llusia and Penuelas, 1998), whereas mild drought might increase emissions (Staudt et al. 2008).

Production and emission of BVOCs could increase in the future due to the increase of productivity and increasing biomass as a result of the rising atmospheric $CO_2$ concentration, although opposite results have also been observed (Penuelas and Staudt, 2010 and references therein).

Tropospheric ozone concentrations are also expected to increase in the coming years (IPCC, 2014). Both increase and decrease of BVOC emissions has been observed under elevated ozone concentration. However, reported results showed an overall increase rather than a decrease (Penuelas and Staudt, 2010 and references therein).

Increase in surface level UVB radiation might substantially increase BVOC emissions (Tiiva et al., 2007; Harley et al., 1996), although it may depend on plant and BVOC species, and on the level of stress (Blande et al., 2009; Harley et al., 1996).

## 1.6 BVOC sampling approaches

To determine which BVOCs are exchanged between the atmosphere and plants and/or soil, and at what rates, several sampling approaches at different temporal and spatial scales are used by the scientific community. The most commonly used methods are shown in figure 1.4.

Leaf, branch and small scale grassland/soil BVOC sampling are performed by using leaf, branch and soil cuvettes or enclosures. A comprehensive review of BVOC sampling systems at the leaf or branch scale has been given by Ortega and Helmig (2008). These enclosures could be either dynamic (with continuous air flow through the enclosure) or static (no air is allowed to circulate) depending on the purpose of the investigation (investigation of VOC flux, examining the activity of VOC-producing enzymes or VOC-substrate concentration), but mostly dynamic enclosures are used for BVOC studies. The inside area of an enclosure ranges between 0.01-10 $m^2$ (Hewitt et al., 2011). Leaf cuvettes are small in area and volume compared to branch and soil enclosures. These leaf and branch measurement techniques are

useful to understand fluxes of trace gases from a leaf or leaves of a plant or a particular plant only by excluding fluxes from soil and other vegetation. Soil cuvettes are useful for measuring VOC fluxes from small soil area, grassland and soil litter. These techniques are also useful to find out the effects of an environmental parameter or biotic stress upon applying it on a single plant or several plants on a small area.

Sampling BVOC at the canopy scale is carried out by using tower-mounted micrometeorological techniques (Karl et al., 2001b). These techniques measure trace gas exchanges between plant canopies and the atmosphere without disturbing the ecosystem and have the potential to be used over long periods. The surface area covered (10000 $m^2$–1 $km^2$) by this sampling technique depends on the height of the canopy and tower, wind speed and atmospheric stability (Hewitt et al., 2011). Among those techniques eddy covariance is the most direct one.

Finally various strategies have been deployed to measure landscape-level fluxes of VOCs, either from the air using airborne eddy covariance (Muller et al., 2014) or from space (Palmer et al., 2006). Air borne landscape-level micrometeorology techniques have low temporal coverage because of high expense, but they are characterized by a high spatial coverage. On the other hand, VOC concentration measurement using earth observation techniques have high temporal and spatial coverage. These measurement techniques are useful to study VOC on a regional or global scale.

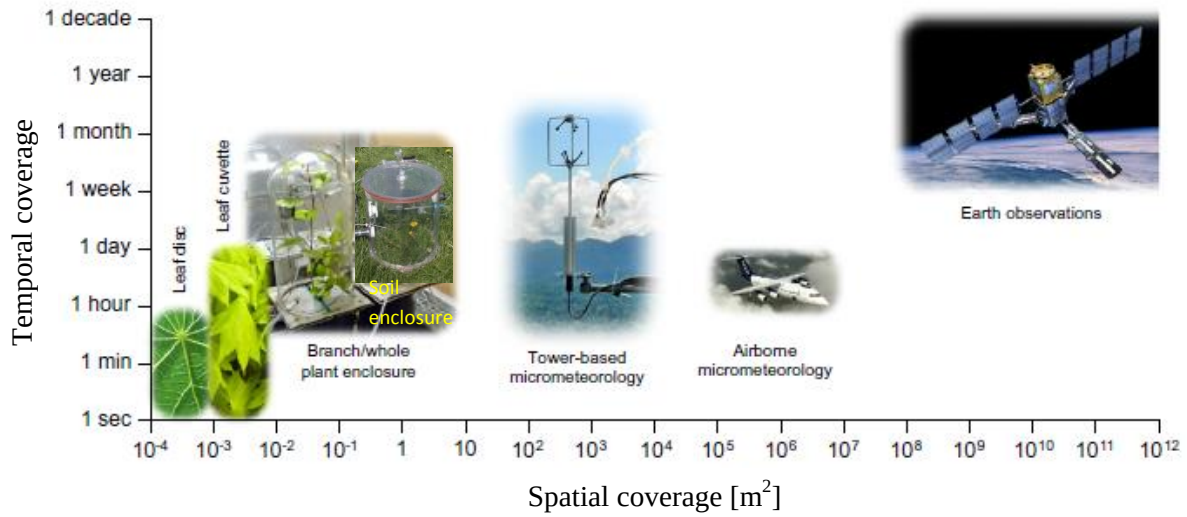



Figure 1.4: The most commonly used VOC sampling approaches with corresponding spatial and temporal coverage (this figure is adapted from Hewitt et al., 2011).

## 1.7 Modelling approaches to estimate BVOC fluxes from plants

In order to quantitatively assess the impact of BVOC emissions on present and future air quality and climate by using regional or global chemistry and climate models, people already started to develop numerical models to estimate those emissions in terms of the driving parameters in the eighties and nineties of the previous century (Tingey et al., 1981; Guenther et al., 1993; Guenther et al., 1995). In what follows we will give a brief introduction on existing leaf-level models which have been used in this thesis. Finally we will briefly indicate how empirical leaf level models can be upscaled to canopy, ecosystem, regional or global level.

### 1.7.1 Leaf scale BVOC emission models

#### 1.7.1.1 Empirical models

Empirical algorithms are widely used in the atmospheric chemistry community to estimate leaf scale BVOC emissions. The first algorithms were developed to describe the light-independent temperature-controlled evaporation of monoterpenes from storage structures in conifers (Tingey, 1981, Guenther 1991, Guenther 1993). The monoterpene emission rate M was found to vary exponentially with temperature T, as shown in Eq. (1.1) where $M_S$ is the monoterpene emission rate at standard temperature $T_S$ (303 K), and $\beta$ (0.09K$^{-1}$) is an empirical coefficient.

$$M = M_S \cdot exp\,(\beta(\mathrm{T} - T_S)) \tag{1.1}$$

In the early nineties, it was found that the emissions of some other compounds, like isoprene were both light- and temperature dependent. According to Guenther et al. (1993), the isoprene emission rate can be estimated by the following equation:

$$I = I_S \cdot C_L \cdot C_T \tag{1.2}$$

where $I_S$ is the isoprene emission rate at standard temperature $T_s$ (303 $K$) and PPFD (1000 µmol m$^{-2}$ s$^{-1}$) conditions, also known as the standard emission factor (SEF), and $C_L$ and $C_T$ (Eq. 1.2) are the emission response functions to variations in light and temperature, respectively, normalized to standard conditions. The functional forms $C_L$ and $C_T$ are given by Eq. 1.3 and Eq. 1.4, respectively. In those equations, $\alpha$ (=0.0027) and $c_{L1}$ (=1.066), $C_{T1}$ (95000 J mol$^{-1}$), $C_{T2}$ (230000 J mol$^{-1}$), and $T_M$ (314 K) are all empirical coefficients which were determined by nonlinear best fit procedures using emission measurements from

different plant species (Guenther et al., 1993), and $R$ is the universal gas constant (8.314 J $K^{-1}$ $mol^{-1}$).

$$C_L = \frac{\alpha c_{L1} PPFD}{\sqrt{1 + \alpha^2 PPFD^2}} \tag{1.3}$$

$$C_T = \frac{\exp \frac{c_{T1}(T - T_s)}{RT_s T}}{1 + \exp \frac{c_{T2}(T - T_M)}{RT_s T}} \tag{1.4}$$

The $C_L$ and $C_T$ response functions are shown in Fig. 1.5. Whereas the hyperbolic shape of $C_L$ is similar to the response function of photosynthesis to PPFD, the shape of $C_T$ reflects the response of enzymatic activity to temperature variations (Guenther et al., 1993). The function goes through a maximum above which enzyme degradation starts to occur.

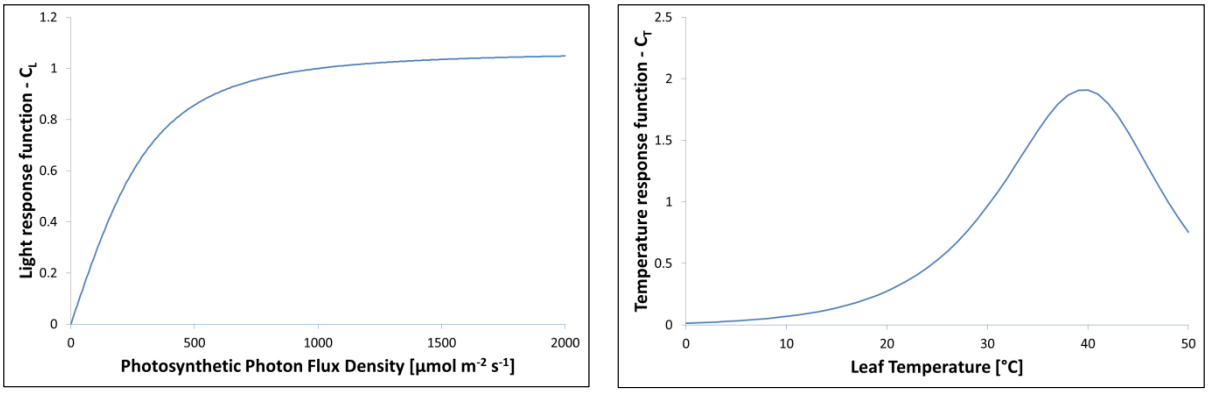



Figure 1.5: Response of the emissions to variations in PPFD (left) and Leaf temperature (right), normalized with respect to standard conditions of 1000 $\mu$mol $m^{-2}$ $s^{-1}$ and 30 °C, respectively.

In recent years, it has become increasingly evident that, apart from instantaneous PPFD and temperature, additional factors such as soil moisture, leaf age and light and temperature history can all modify emission rates and response functions, obtained from experimental data, have been incorporated in MEGAN, the Model of Emissions of Gases and Aerosols from Nature (Guenther et al., 2006).

Over the years it has also become clear that, in contrast to non-stored monoterpenes or to isoprene, several compounds show emissions both in the absence and in the presence of light.

This is for instance the case for methanol. The leaf level emission for those compounds is then given as the product of a standard emission factor $\varepsilon_{leaf}$ multiplied by $\gamma_{PT}$, the combined response function of the emissions to light and temperature (Stavrakou et al., 2011):

$$\gamma_{PT} = (1 - LDF) \cdot \gamma_{T-li} + LDF \cdot \gamma_P \cdot \gamma_{T-ld} \quad (1.5)$$

where LDF (0.80 for methanol) is the light-dependent fraction of the emission at standard conditions, $\gamma_P$ is the light response of methanol emissions, $\gamma_{T\text{-}ld}$ is the temperature response for the light-dependent fraction of the emissions, $\gamma_{T\text{-}li}$ is the temperature response for the remainder of the emissions that are not light dependent. The temperature response for the light-dependent fraction of the emissions $\gamma_{T\text{-}ld}$ is estimated as

$$\gamma_{T-ld} = E_{opt} \cdot [C_{T2} \cdot e^{C_{T1} \cdot x} / (C_{T2} - C_{T1} \cdot (1 - e^{C_{T2} \cdot x}))] \quad (1.6)$$

where for methanol, $E_{opt}$ is 1.61, $x=[(1/T_{opt})-(1/T)]/0.00831$, $T$ is the leaf temperature, $T_{opt}$ is 313 K, $C_{T1}$ and $C_{T2}$ are 60 and 230, respectively .

The temperature response for the light independent fraction of the emissions is estimated as

$$\gamma_{T-li} = \exp(\beta(T - 303)) \quad (1.7)$$

where $T$ is the leaf temperature and $\beta$ is the temperature response factor (0.08 $K^{-1}$ for methanol).

The $\gamma_{PT}$ response function has been used in the present work as a methanol production function to estimate methanol emissions from maize leaves with the dynamic BVOC emission model of Niinemets and Reichstein (see chapter 3).

### 1.7.1.2 Dynamic emission models

To account for temporary storage of hydrophilic low vapour pressure compounds in a non-specific aqueous pool in the leaf before diffusion through the stomata, Niinemets and Reichstein (2003a and 2003b) have developed a dynamic BVOC emission model, which was later extended for non-specific storage into a lipid leaf pool (Noe et al. 2006). According to this model BVOC emissions are not only determined by the production rate, but also by the

physicochemical properties of the compounds which can strongly impact their transfer from the site of synthesis to the site of emission.

A schematic representation of the transfer of BVOCs from the site of synthesis to the atmosphere is depicted in Fig. 1.6. After biosynthesis in the leaf, BVOCs can either be directly transferred to temporary non-specific aqueous or lipid pools (depending on their physicochemical properties) or be stored in specific storage pools, such as glandular trichomes or resin ducts. Compounds from those specific pools may eventually be transferred to the aqueous or lipid pool as well. From the temporary pools, BVOCs are transferred to the gaseous pool which includes the sub-stomatal cavities. The BVOCs are finally transported from the gaseous pool to the atmosphere by diffusion through the stomata or, to a much smaller extent, through the cuticular layer (Niinemets et al., 2004, Noe et al., 2006).

The pool sizes strongly depend on the physicochemical characteristics of the BVOCs. Whereas lipophilic compounds with a high octanol-to-water partition coefficient (e.g. non-oxygenated terpenes) can be stored in significant quantities in the lipid phase of the leaf, hydrophilic compounds with a low Henry's law coefficient (e.g. alcohols and acids) are easily stored in the aqueous phase of the leaf. Compounds for which the pool sizes are very small (e.g. isoprene) will be released immediately after production.

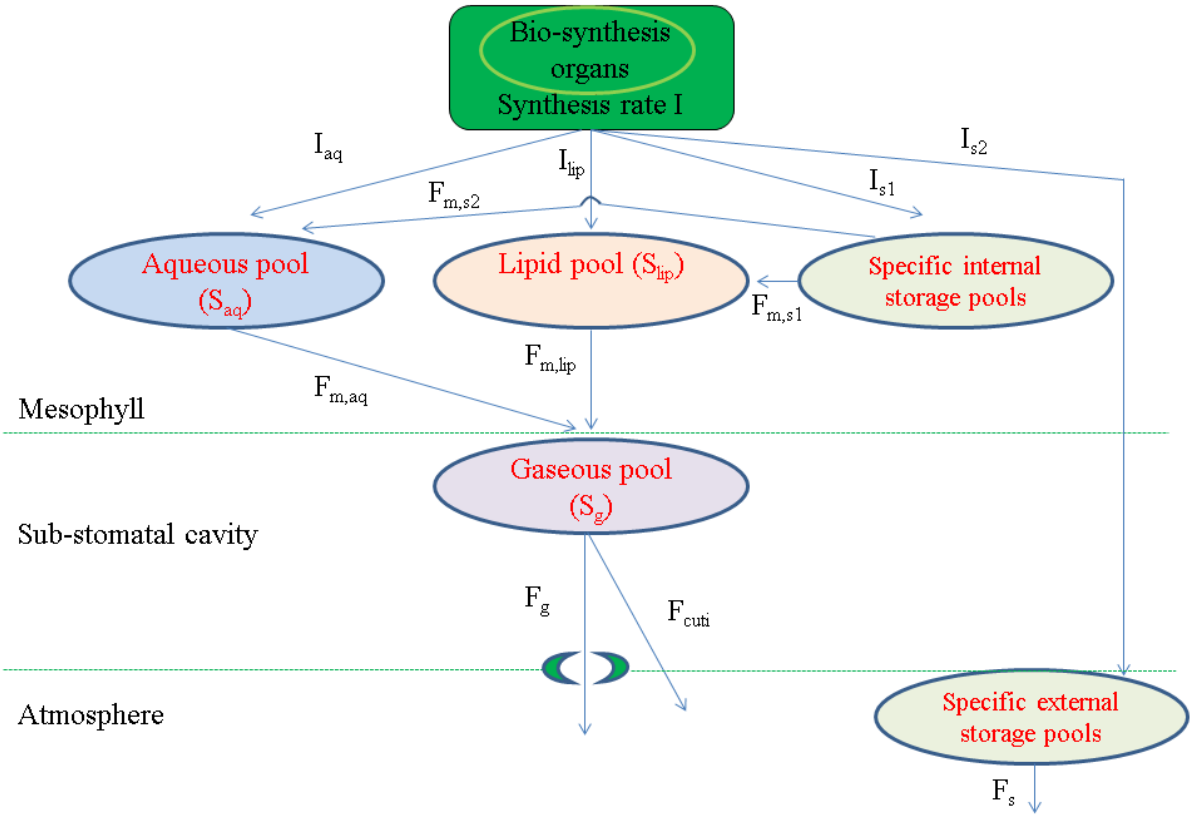



Figure 1.6: Schematic representation of the transfer of BVOCs from the site of synthesis to the atmosphere. The compounds for which there are no specific storage organs are transferred from the site of synthesis to the atmosphere through a series of membrane bilayers, non-specific aqueous ($S_{aq}$) and lipid pools ($S_{lip}$), the gaseous pool ($S_g$), and the stomata and/or the cuticular layer. The compounds which are stored in the specific storage pools follow the same way before being emitted to the atmosphere. In this diagram, $F_{m,aq}$ is the flux from the aqueous to the gaseous pool, $F_{m,lip}$ is the flux from the lipid to the gaseous pool, $F_{m,s1}$ is the flux from storage organs to the lipid pool, $F_{m,s2}$ is the flux from storage organs to the aqueous pool, $F_g$ is the flux through the stomata, $F_{cuti}$ is the flux through the cuticular layer, and $F_s$ is the flux from the specific external storage pools (this diagram is drawn based on information from Niinemets et al., 2004, and Noe et al., 2006).

In this dissertation the original two-pool model of Niinemets and Reichstein (2003a and 2003b) has been validated against methanol emission measurements from maize leaves (see chapter 3). In this model, the emission of a compound is given by $F_g=G_s\,((p_i-p_a)/P)$, where $G_s$ is the stomatal conductance, $p_i$ and $p_a$ are the partial pressures of that compound in the intercellular air space of the leaf and in the air, respectively, and $P$ is the air pressure. For

highly volatile compounds like isoprene, reduction in $G_s$ has no effect on $F_g$. Indeed, as isoprene does hardly partition in the aqueous phase this will lead to an immediate increase of $p_i$ which compensates the reduction of $G_s$ (assuming that the production rate is constant). Therefore, a new steady state between pools will be established within seconds after a perturbation in $G_s$. However, if the compound is highly water soluble like methanol or acetic acid, the increase of $p_i$ will be slow and the establishment of a new steady state will be delayed. A reduction of $G_s$ will therefore lead to a temporary reduction in $F_g$ which will last until a new equilibrium condition between the gas and liquid BVOC pools has been established. The dynamics of the gaseous and liquid pool sizes of the volatiles is governed by the following differential equations (Niinemets and Reichstein, 2003a and 2003b):

$$\frac{dS_g}{dt} = F_{m,aq} - F_g \tag{1.8}$$

$$\frac{dS_{aq}}{dt} = I_{aq} - F_{m,aq} \tag{1.9}$$

where $S_g$ and $S_{aq}$ are the sizes of the gas and liquid pools, respectively, $I_{aq}$ is the synthesis rate of the volatile, $F_g$ is the diffusion flux through the stomata and $F_{m,aq}$ is the diffusion flux from the site of synthesis to the outer surface of the cell walls. By solving this set of coupled differential equations and taking into account physicochemical BVOC properties and specific plant physiological properties, BVOC fluxes can be estimated when assuming a pre-determined BVOC production function and using instantaneous values of the stomatal conductance. More details can be found in the original papers of Niinemets and Reichstein (2003a and 2003b) and in the annex to chapter 3 of this dissertation.

### 1.7.2 From leaf scale to global scale

The leaf level algorithms described in the previous paragraphs are vital elements in models which are used to estimate emissions from terrestrial ecosystems at ecosystem scale and, by extension, to regional and global scale. The Model of Emissions of Gases and Aerosols from Nature (MEGAN) has been developed by Guenther et al. (2006) for this purpose and has recently been upgraded (Guenther et al, 2012). Several vegetation types are considered (plant functional types) and the model accounts for regional variation in standard emission factors by using geographically gridded databases of emission factors for each plant functional type. Emissions ($F_i$) of a chemical species or class of species $i$ from terrestrial vegetation in a surface grid cell are given as the product of the emission activity factor $\gamma_i$ with the sum over

all vegetation types of the product of the emission factor at standard conditions $\varepsilon_{i,j}$ and the fractional grid box areal coverage $\chi_j$ of vegetation type $j$ (Guenther et al, 2012):

$$F_i = \gamma_i \sum_j \varepsilon_{i,j} \aleph_j \tag{1.10}$$

The emission activity factor $\gamma_i$ accounts for the response of the emissions to variations of the leaf area index (LAI), and to variations in light ($\gamma_{P,i}$), temperature ($\gamma_{T,i}$), leaf age ($\gamma_{age,i}$), soil moisture ($\gamma_{SM,i}$) and $CO_2$ inhibition ($\gamma_{C,i}$):

$$\gamma_i = C_{CE} LAI \gamma_{P,i} \gamma_{T,i} \gamma_{age,i} \gamma_{SM,i} \gamma_{C,i} \tag{1.11}$$

The different response functions are normalized with respect to standard conditions and the canopy environment coefficient $C_{CE}$ is assigned a value that results in a $\gamma_i$ value of 1 in standard conditions.

By summing the emissions $F_i$ over the appropriate grid cells, the MEGAN model allows to estimate BVOC fluxes from ecosystem scale to regional and even global scale. Detailed information on the MEGAN model and the functional forms of the different response functions can be found in the literature (Guenther et al., 2006, Stavrakou et al., 2011, Guenther et al. 2012).

## 1.8 BVOC fluxes from agricultural plant species

According to the World Bank, around 38 % of the earth land surface is agricultural land whereas 31 % of the land surface is made up of forests (The World Bank, 2017). Despite this, forests have been the most widely studied ecosystem for BVOC fluxes (see Niinemets et al., 2013 for a review) whereas only a limited number of studies have focused on agricultural croplands (Karl et al., 2005; Das et al., 2003; Bachy et al., 2016; Brunner et al., 2007; Davison et al., 2007; Brilli et al., 2012; Ruuskanen et al., 2011; Hortnagl et al., 2011; Bamberger et al., 2010; Graus et al., 2013). Despite being the highest cultivated crop species in the world (FOA, 2014), information about BVOC fluxes from wheat (*Triticum aestivum* L.) is rare and confined to only a few studies so far (Wenda-Piesik, 2011; Hartikainen et al., 2012; Piesik et al., 2010). Maize (*Zea mays* L.) is the second most important crop in the world in terms of cultivated area (FOA, 2014). Nevertheless, only a few studies on maize BVOC exchanges are available in the literature (MacDonald and Fall, 1993; Das et al., 2003; Graus et al., 2013; Bachy et al., 2016). Moreover, except for a recent field study by Bachy et

al. (2016), all studies were performed for a limited period and didn't cover all the developmental stages of maize. To develop a standard emission factor (SEF) of BVOCs for a particular plant species, understanding of emissions for all developmental stages is necessary. Compared to wheat and maize, more studies have been performed on grassland, another important agricultural ecosystem (Warneke et al., 2002; de Gouw et al., 1999; Karl et al., 2001 a; Karl et al., 2001 b; Custer and Schade, 2007; Brunner et al., 2007; Davison et al., 2007; Brilli et al., 2012; Ruuskanen et al., 2011; Hortnagl et al., 2011; Bamberger et al., 2010; Jaars et al., 2016). Although a number of studies have been performed on grassland under control and abiotic stress conditions, no investigations on grazing-induced BVOC emissions have been reported yet. Information about BVOC fluxes from other crop species is rare as well. A few studies on biofuel plant species have also been performed (Graus et al., 2013; Eller et al., 2011; Copeland et al., 2012; Crespo et al., 2013). Due to the lack of data/information on agricultural crop species, Karl et al. (2007) used default SEF values for agricultural crops obtained from other ecosystems when producing a European plant-specific emission inventory of BVOC and they pointed out the necessity of further experiments on agricultural crop species. In addition, SEF values estimated by Bachy et al. (2016) for a C4 crop species (maize) are several orders of magnitude lower than the SEF values used in the models by Guenther et al. (2012), Lathiere et al. (2006), and Stavrakou et al. (2011). Because of the abovementioned reasons, more studies related to BVOC fluxes for agricultural plant species are necessary.

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# Chapter 2: Objectives and methodology

## 2.1 Objectives

The work presented in this thesis was carried out in the framework of a collaborative research project CROSTVOC (CROp Stress VOC) between the BIODYNE (Biosystems Dynamics and Exchanges) and Plant Biology Laboratory research groups at the University of Liege and the Royal Belgian Institute for Space Aeronomy, and funded by FNRS (Fonds National de la Recherche Scientifique, research grant ID. T.0086.13). The project focuses on BVOC fluxes from agricultural crop species as far less studies have been performed on agricultural crop (graminoid) species than on woody (tree) plant species. To improve the knowledge about the biosphere-atmosphere exchanges of BVOCs for agricultural crop species, 3 specific types of vegetation, maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and grassland, were considered in the CROSTVOC project. These 3 model systems were chosen because of their high cultivation in Belgium. Moreover, around 13 and 15% of the global agricultural land is cultivated with maize and wheat, respectively, and a quarter of the earth's surface is grassland (FAO, 2014).

The investigations have been performed both under natural conditions in the field using micrometeorological and enclosure measurement techniques and under controlled conditions in an environmental chamber using the enclosure measurement technique. BVOC fluxes were measured from a maize field for a whole growing season using the micrometeorological measurement technique (Bachy et al., 2016), which is suitable for measuring BVOC fluxes at field or ecosystem level. However, it was not straightforward to separate BVOC fluxes from the vegetation and soil, and to understand the drivers of emissions from the data obtained using this technique as the meteorological parameters covary and cannot be controlled in an ecosystem. Therefore, BVOC fluxes from the same maize variety were also measured using the enclosure technique in an environmental chamber, where environmental parameters could be controlled and fluxes were measured only from plant shoots or leaves. BVOC fluxes were followed from young to senescent leaves since there is no information about BVOC fluxes from senescent maize leaves yet in the literature. As maize leaves senesce throughout the growing season, fluxes from senescent leaves could provide a significant contribution to the total BVOC budget from a maize field. According to a previous study on maize (Bachy et al., 2016), methanol is the highest emitted compound among all emitted BVOCs. Moreover, the little information available in the literature on BVOC emissions from maize (MacDonald and

Fall, 1993; Das et al., 2003; Graus et al., 2013) indeed indicates that it could be an important plant species for exchanging methanol with the environment. Thus, in this investigation, methanol will be the main focused compound among all exchanged BVOCs.

In addition to a lack of data on BVOC fluxes from highly cultivated/abundant crop species, large uncertainties in BVOC flux inventories are attributed to stress-induced emissions as well. Therefore the CROSTVOC project also focussed on the impact of stress on BVOC emissions from a managed grassland ecosystem. Both an abiotic ($O_3$) and a biotic (cow grazing) stress factor were considered. In order to investigate the effect of enhanced oxidative stress long-term BVOC and $O_3$ flux measurements have been performed with the eddy covariance measurement technique. Assessing the effect of grazing on the emissions from the eddy covariance flux measurements was not straightforward as it was not possible to measure simultaneously BVOC fluxes from a grazed and undisturbed (reference) grassland under similar meteorological conditions (as only one BVOC analyser was available). Therefore, the micrometeorological flux measurements on the grassland ecosystem were complemented by dedicated dynamic enclosure field experiments where cows were let to graze a small area of grassland for a short time and BVOC fluxes from this grassland and from ungrazed grassland were subsequently measured continuously for a few consecutive days (see § 5.2)

Within the framework of the CROSTVOC project, the specific research objectives of this dissertation were:

I. to investigate BVOC fluxes from different developmental stages of maize leaves under controlled environmental conditions in order to answer the following questions: (i) How do BVOC flux intensity (absolute flux values), pattern (responses to environmental conditions) and spectra (compound composition) vary among the different leaf developmental stages of maize? (ii) What are the controlling parameters of methanol fluxes for maize leaves? and (iii) what are the contributions of the different developmental stages to the total methanol emission by a maize leaf/plant ?
II. to study BVOC fluxes from a managed grassland in order to answer the following questions: (i) what are the main exchanged BVOCs from undisturbed grassland and their exchange intensity and main controlling parameters? (ii) how do grazing-induced BVOC emissions differ from the constitutive emissions both qualitatively (composition) and quantitatively (intensity)? (iii) how do BVOC fluxes vary between

two grassland management practices: rotational grazing in the present study and seasonal grassland harvest previously reported in the literature?

## 2.2 Methodology

To fulfil the abovementioned objectives, BVOC fluxes together with $CO_2$ and $H_2O$ vapour fluxes were measured from maize and grassland. Leaf scale flux measurements were performed for the investigations on maize, and soil cuvette scale flux measurements were performed for the investigations on grassland. A brief description of the followed experimental procedure is given below.

### 2.2.1 Experimental setup

Investigations undertaken to fulfil the first objective were performed in an environmental chamber located at the Royal Belgian Institute for Space Aeronomy. A picture of enclosed vegetation inside the environmental chamber is shown in Fig. 2.1. Details about the enclosure system and the environmental chamber are given in § 3.2.1 and § 3.2.2. A schematic representation of the environmental chamber setup with other accessory equipment for air sampling and analysis is illustrated in Fig. 2.2.

Air is supplied to the enclosed plant biomass in the environmental chamber by a compressor with drying function (DK50 2×2V/110 S/M/230 V, EKOM, Germany). The reduction of $CO_2$ concentration and relative humidity (due to compression and drying) in the supplied air are adjusted by controlled addition of $CO_2$ from a gas bottle and addition of moisture by sending a controlled portion of the total air flow through two glass bottles filled with deionised water. Air with adjusted $CO_2$ concentration (~400 ppmv) and relative humidity (~40%) is then scrubbed of VOCs by two sets of active charcoal filters (Airpel 10 and Organosorb 10-CO, Desotec, Belgium) and filtered for particles (Zefluor PTFE Membrane Filter, 2 µm pore size, Pall Laboratory, Ann Arbor, MI, USA) before being distributed at equal flow rates (measured by mass flow meters) to the different enclosures. Air sampled from the enclosures is pumped through PFA (perfluoroalkoxy Teflon) tubing towards a manifold which is located outside the environmental chamber. Each tube is fixed to a three-way PFA solenoid valve (Valcor Engineering Corporation, Springfield, NJ, USA), and by sequentially actuating one valve out of the set of six, sampled air from the corresponding enclosure is directed towards the LI-7000 infrared gas analyser (LI-COR, Lincoln, Nebraska, USA) and the PTR-MS (Proton Transfer Reaction Quadrupole Mass

Spectrometer, Ionicon Analytik G.m.b.H, Innsbruck, Austria) for $CO_2$/$H_2O$ and VOC analysis, respectively. An air flow is continuously maintained through the tubes from the other five enclosures by directing the output of the corresponding three-way valves towards an exhaust line by means of a membrane pump (MVP-055, Pfeiffer Vacuum, Germany). All the sampling lines from the enclosures to the analysing instruments are heated and thermally insulated to avoid condensation.

The calibration of the PTR-MS for BVOC analysis is performed by supplying a gravimetrically prepared mixture of different BVOCs in nitrogen (Apel-Riemer Inc., Denver, CO, USA) to the PTR-MS after dilution with zero air generated by a catalytic converter (Parker® ChromGas® Zero Air Generator, model 1001, Parker Hannifin Corporation, Haverhill, MA, USA). A membrane pump is supplying air to the catalytic converter through a set of active charcoal filters. A dew point generator (LI-COR 610, LI-COR, Lincoln, Nebraska, USA) is used to change the humidity in the supplied air from the membrane pump in order to calibrate the PTR-MS for water vapour analysis.

A data logger (Data Acquisition / Switch Unit 34970A, Santa Clara, CA, USA) is collecting the analog output signal of the infrared gas analyser, the output signals of all the temperature and relative humidity sensors in the enclosures and temperature and light sensors in the environmental chamber, and the mass flow rates of the purge air supplied to the individual enclosures. The manifold and the calibration system (3-way-valves, flow controllers), as well as the communication with the datalogger are computer-controlled using Labview® software. A laptop is used to control the operation of the PTR-MS and store all the analysed data.

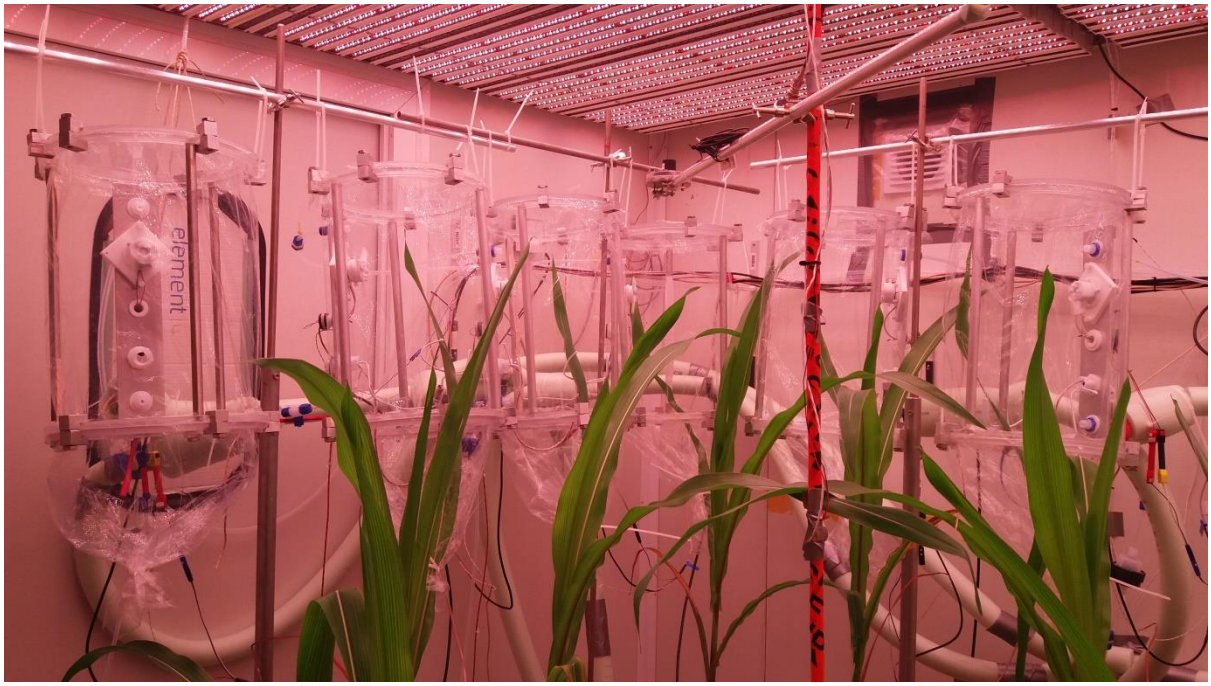

Figure 2.1: Enclosed maize leaf in the flow-through enclosures inside the environmental chamber.

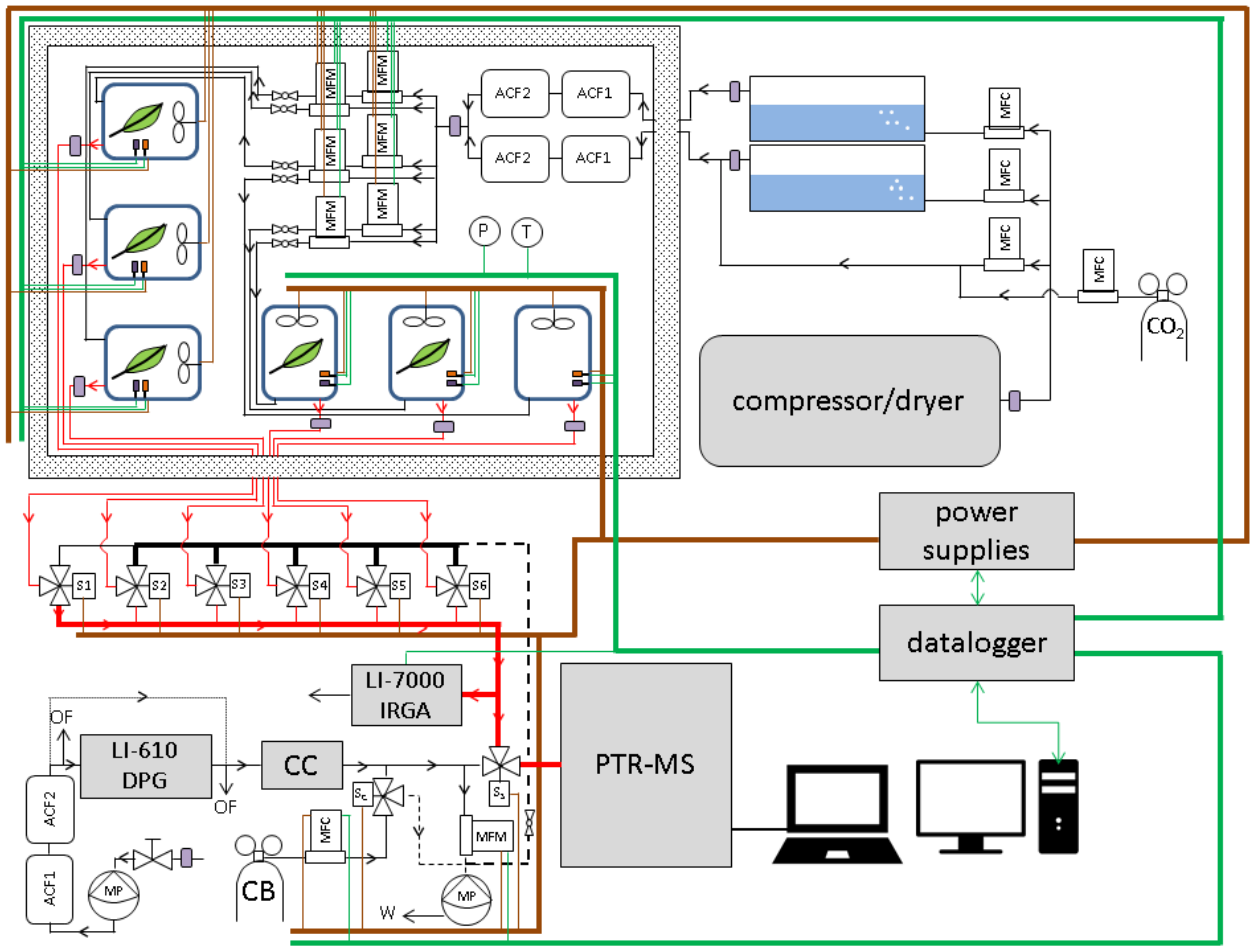


Figure 2.2: Schematic representation of the environmental chamber setup with the other accessory equipments for air sampling and analysis. In this schematic diagram, the rectangle with patterned border represents the environmental chamber, $CO_2$: $CO_2$ gas bottle, MFC: mass flow controller, ACF: active charcoal filter, MFM: mass flow meter, P: light sensor, T: temperature sensor, S: solenoid

valve, IRGA: infrared gas analyzer, DPG: dew point generator, CC: catalytic converter, CB: calibration gas bottle, MP: membrane pump, W: waste line, OF: overflow. All the red lines represent heated tubes and all the purple rectangles represent teflon filters.

Investigations regarding the second objective were performed in a managed grassland at the Dorinne Terrestrial Observatory (DTO) in Namur, Belgium. A picture of the DTO site is shown in Fig. 2.3 and a detailed description about the DTO site is given in § 5.2.1. A schematic representation of the experimental setup at the DTO with other accessory equipment for air sampling and analysis is illustrated in Fig. 2.4.

Grassland/atmosphere gas exchange was determined by using cylindrical automated dynamic flow-through enclosures (n=6, Figure 2.5) which are of similar design as those described by Pape et al. (2009). Opposite to the investigations in the environmental chamber, ambient air was supplied to the dynamic flow-through enclosures. Each enclosure was 43 cm high and had an inner diameter of 35 cm, resulting in an enclosed surface area of 962 $cm^2$ and a headspace volume of 41.3 L. The outer supporting frame, consisting of two rings held together by four vertical plates, and the moveable lid of the enclosure were made of 10 mm thick polycarbonate plate (Eriks, Hoboken, Belgium). The frame held a thin (50 µm), chemically inert PFA (Perfluoroalkoxy Teflon, Norton, Saint-Gobain Performance Plastics, NJ, USA) sheet which is 96% transparent to photosynthetically active radiation. The inner surface of the lid was also covered by a PFA sheet which was fixed to the lid by a silicone O-ring (Eriks, Hoboken, Belgium). A waterproof, custom-made aluminium box containing a DC motor with transmission (Robert Bosch GmbH, Karlsruhe, Germany) was mounted to the outer frame of the enclosure to control the lid position by a lever arm. The angular position of the lid was monitored by a Hall-effect inclinometer (type UV-00H-SW2, Pewatron AG, Zürich, Switzerland) which was fixed to the lever arm. Each enclosure was clamped to a pre-installed aluminium collar, which was partially buried in the soil (burial depth of ca. 5 cm) to avoid leaks. The part of the collar which was in contact with the air in the enclosure was covered by PFA sheet to avoid BVOC losses to the metal surface. The headspace volume above the enclosed grass was around 46 l including the collar volume. Ambient air was introduced through an opening in one of the vertical supporting plates of the enclosure at a height of 12 cm from the bottom of the enclosure. A similar opening at a height of 32 cm was provided in the opposite plate allowing the majority of the incoming air to leave the enclosure

when the lid was closed. This plate also served to support a PFA bulkhead to fix a ¼" inch PFA tube through which air was sampled from the centre of the enclosure at a height of 26 cm. The air inside the enclosure was thoroughly mixed by a waterproof fan (Conrad Electronics Benelux BV, Mortsel, Belgium) with custom-made Teflon housing and blades which was mounted on the support plate above the inflowing air entrance at a height of 26 cm. A temperature sensor (RTD-3-F3105-60-T, Omega Engineering Inc., Norwalk, CT, USA) with Teflon coated lead wires was positioned in the centre of the enclosure, close to the sampling point, and a relative humidity sensor (type HIH400-003, Honeywell, Golden Valley, MN, USA) was located in the Teflon interface piece at the exit of the enclosure air.


Figure 2.3: Grassland patches (1 and 2) in the DTO site separated by barbwire. Three automated soil enclosures in patch 1 and 3 in patch 2 are installed for the measurement.

A polyester box, the content of which is shown in the inset of Figure 2.5, was associated to each enclosure and was positioned at a lateral distance of about 10 cm from the enclosure. Each box contained an axial fan (Micronel, Zürich, Switzerland) to supply an ambient ingoing air flow (60-80 L $min^{-1}$) through a 32 mm outer diameter PVC (Poly Vinyl Chloride) tube from a height of about 50 cm above the surface. This height was chosen to avoid a large difference in composition between the air flowing into the cuvette and the air close to the biomass outside of the cuvette. This large ingoing flow resulted in a BVOC residence time in the enclosures of 34 to 46 s. A mosquito net at the inlet prevented the sampling of larger particles or insects. The ingoing air flow was measured downstream the fan by an airflow sensor (type AMW720P1, Honeywell, Golden Valley, MN, USA) before being directed

towards the enclosure through POM (polyoxymethylene) and PVC tubing. The air flow sensor was calibrated before and after each field campaign by comparison against a MKS mass flow controller (type 1559A, 100 sLm range, MKS, Andover, MA, USA). To measure backgroung concentation, a portion of the inflowing air was subsampled at 10 cm upstream of the air entrance to the enclosure. Both the inflowing air and enclosure air ¼" PFA sampling tubes were provided with an in-line particle filter (Zefluor® PTFE Membrane filter, pore size 2 µm, Pall Corporation, Ann Arbor, MI, USA) and were connected to a PFA three-way solenoid valve (Valcor Engineering Corporation, Springfield, NJ, USA) located in the box. The exit of the PFA valve was connected to the main 3/8" PFA sampling line which in turn was connected to the gas manifold in the shelter. The shelter is placed closed to the experimental grassland patches and contains all equipment for the experiment except the soil enclosures and the accompanied boxes. The manifold system is exactly the same as the one used in the environmental chamber and the operations for sampled air analysis, instrumental calibration and data collection are very similar to those used in the environmental chamber. Some differences with respect to the set-up in the environmental chamber are the use of a LI-840A infrared gas analyser for both $CO_2$ and $H_2O$ analysis, the application of a separate datalogger (Data Acquisition / Switch Unit 34970A, Santa Clara, CA, USA) for collecting environmental parameter data and instrumental data related to the automated enclosures, and the use of an additional computer to control the operation of the automated enclosures using Labview software. Moreover, instead of charcoal filtered air (which was used in the environmental chamber experiments), ambient air is used to generate zero air by the catalytic converter for instrument calibration purposes.

This main sampling line, of which the length (20 m) was identical for all enclosures, was heated to a few degrees above ambient temperature to prevent condensation, thermally insulated and put in a conduit for protection against rodents. White plastic foil was wrapped around the conduit to protect the tube from overheating by sunlight. Sampled air was pumped towards the gas manifold by a membrane pump at a flow rate of 11-12 standard L $min^{-1}$. This high flow rate ensured a small BVOC residence time of ca. 7 sec in the sampling lines. A set of electrical cables for powering the motor, the fans, the sensors, the valve and for collecting the sensor outputs were provided for each enclosure and, apart from the motor power cable, all electrical connections were established in the polyester box, in which they were protected from rain. The operation of the enclosures and monitoring of the enclosure parameters was automated by Labview® software (National instruments, Austin, TX, USA) through

Labview®-controlled Analog Input (NI 9205), Digital Output (NI 9477 and NI 9478) and Relay Output (NI 9485) modules (National instruments, Austin, TX, USA) and the status of the enclosures and the parameter values were instantaneously visualized on the screen and saved on the hard disk of a computer.

A photosynthetic photon flux density (PPFD) sensor (type LI-190SA, LI-COR, USA) was mounted on an iron bar at the top height level of the enclosures and placed at a central position about 5 m away from the enclosures.

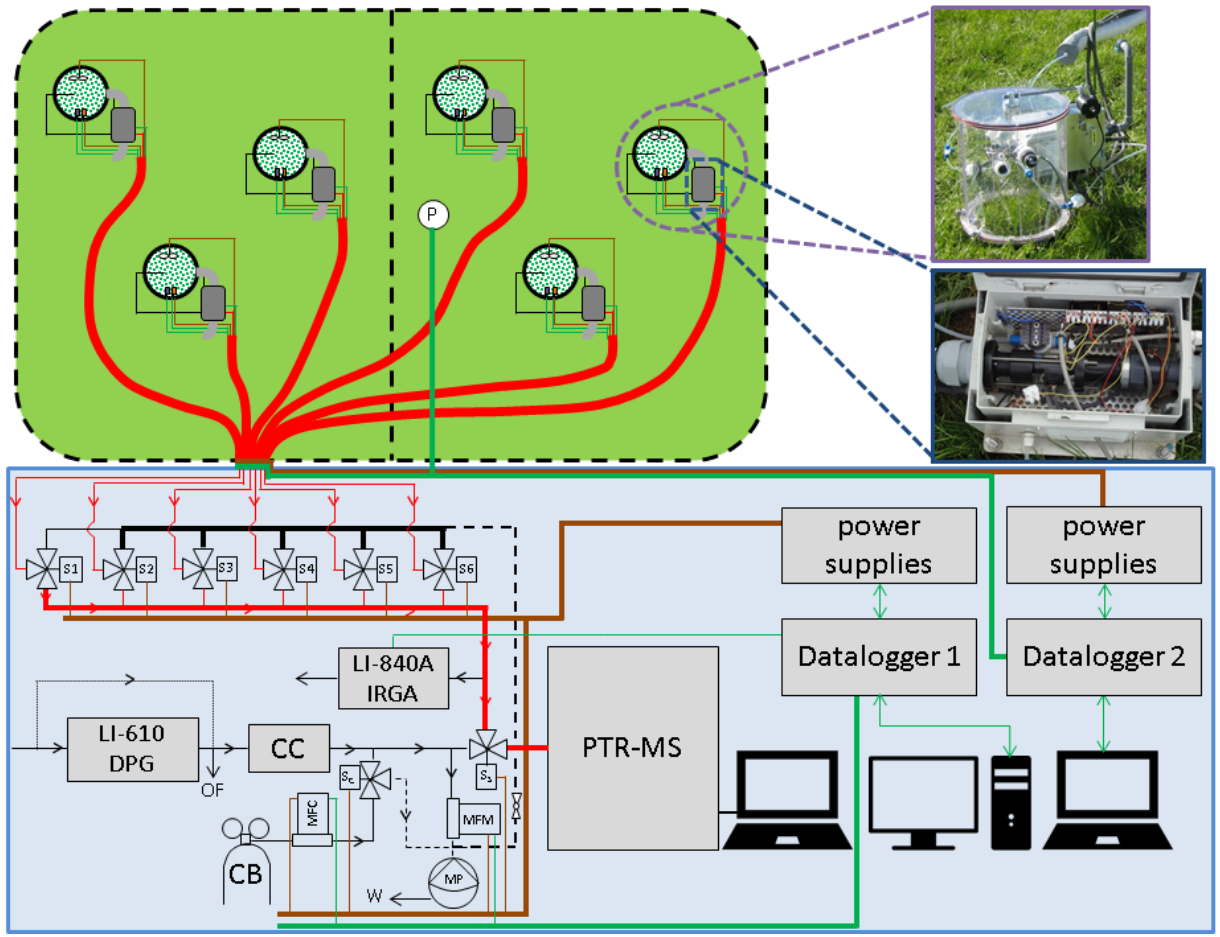


Figure 2.4: Schematic representation of the grassland experiment setup with the other accessory equipments for air sampling and analysis at the Dorinne Terrestrial Observatory (DTO). In this schematic diagram, the green rectangle is the experimental grassland,

enclosed and separated into two patches (grazed and undisturbed), and the light blue rectangle is the shelter. P: light sensor, MFC: mass flow controller, MFM: mass flow meter, S: solenoid valve, IRGA: infrared gas analyzer, DPG: dew point generator, CC: catalytic converter, CB: calibration gas bottle, MP: membrane pump, W: waste line. All the red lines represent heated tubes. All the sampling lines have the same length, the entire sampling lines are not shown in the schematic drawing.

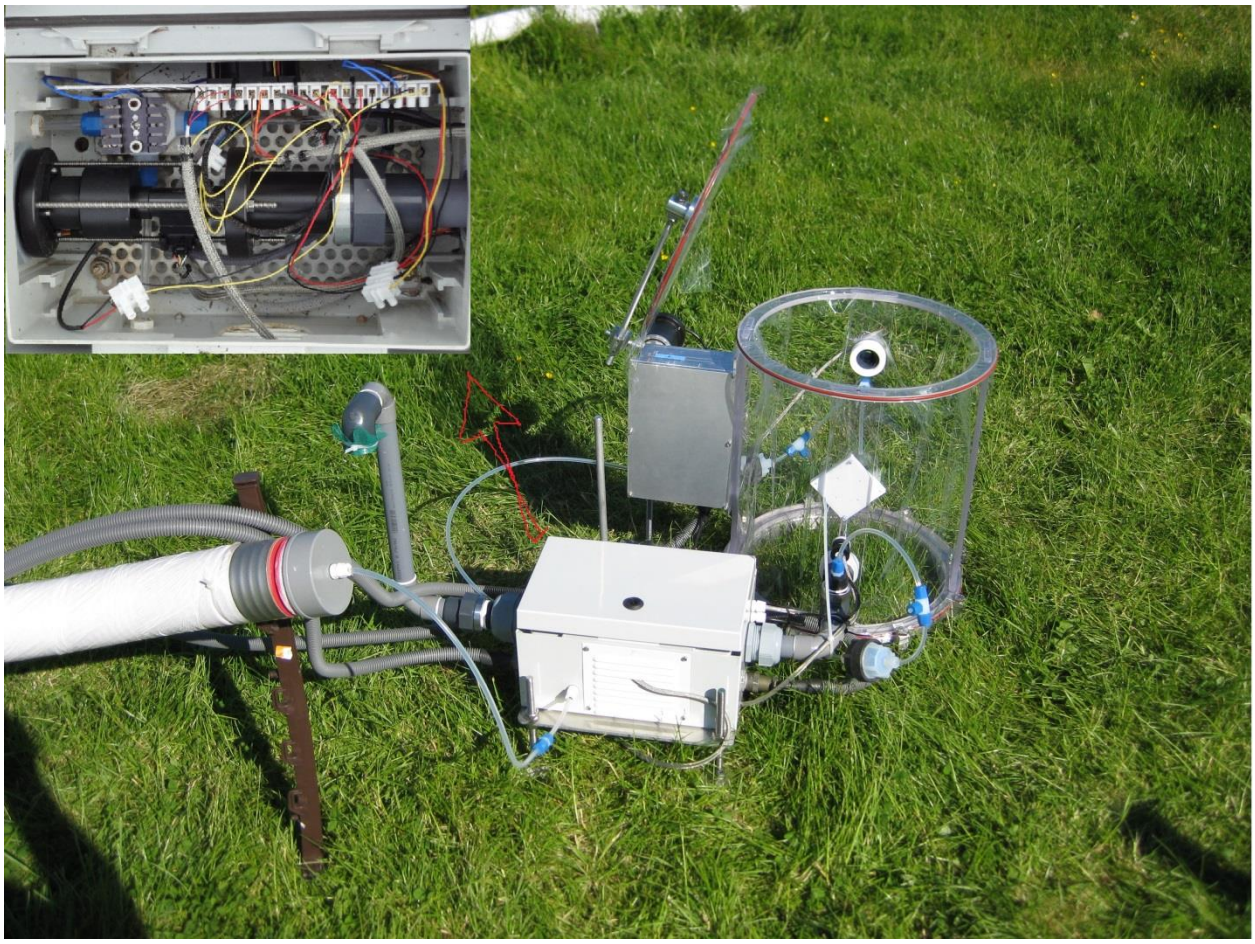

Figure 2.5 : An Automated dynamic flow-through enclosure with ancillary equipment on the grassland. The inset picture shows the interior of the white polyester box which mainly contains the ventilator to produce the ingoing air flow, the flow sensor, and the three-way PFA valve to alternate sampling of inflowing and enclosure air.

### 2.2.2 Sampling technique: dynamic flow-through enclosures and automated dynamic flow-through chambers

Cylindrical dynamic flow-through enclosures were used to enclose plant material and sample BVOC for the experiments performed in the environmental chamber. Depending on the size and shape of the enclosed plant material, several kinds of enclosures were used. Configurations of the dynamic flow-through enclosures used in those experiments are described in detail in their corresponding methods and material sections (see § 3.2.2 and § 4.2.2). In the DTO where sampling was performed on grassland, automated dynamic flow-through chambers were used to enclose grass and soil. These automated dynamic flow-through chambers are already described in § 2.2.1.

### 2.2.3 PTR-MS

BVOC concentrations in the sampled air were measured using a conventional quadrupole-based hs-PTR-MS (Proton Transfer Reaction-Mass Spectrometer, Ionicon Analytik G.m.b.H, Innsbruck, Austria), which allows on-line monitoring of VOCs with a concentration as low as a few pptv (Lindinger et al., 1998). The instrument consists of three main parts: an ion source, a drift tube, and an analysing system (Figure 2.6). In the ion source, primary (reactant) ions (only $H_3O^+$ for these investigations) are produced in a hollow cathode discharge in pure water vapour. VOCs and other volatile reactant gases in the sampled air undergo non-dissociative proton transfer from the primary ions in the drift tube reactor, as shown in equation 2.1. The analysing system of the PTR-MS consists of a quadrupole mass filter in conjunction with a secondary electron multiplier which allows mass separation and detection of the ions, respectively.

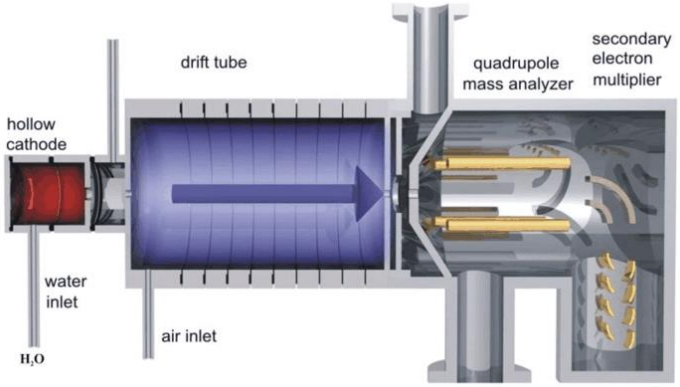


Figure 2.6: Schematic view of the PTR-MS (courtesy: Ionicon).

$$H_3O^+ + VOC \rightarrow VOCH^+ + H_2O \quad (2.1)$$

As $H_3O^+$ ions do not react with any of the major components present in clean air (except $CO_2$, as will be discussed in § 4.2.4.1), air to be analysed could be used as a buffer gas, which makes PTR-MS very sensitive to VOCs in sampled air. Sampled air does not need to be prepared (e.g. preconcentration or drying) before the measurement, hence headspace samples can be introduced directly into the drift tube. Proton transfer in the drift tube is a soft chemical ionization method which keeps fragmentation rates of the nascent excited ionized VOCs low compared to electron impact ionization. More details about the PTR-MS technique are given by Lindinger et al. (1998) and Ellis and Mayhew (2014). The operating conditions and procedure of the PTR-MS for the different experiments carried out in this work are given in detail in the corresponding materials and methods sections (see § 3.2.3, § 4.2.3 and § 5.2.2).

### 2.2.4 GC-MS and Infrared gas analyser

To increase the confidence level on BVOC identification, sampled air from the automated dynamic flow-through chamber at DTO was also analysed using Gas Chromatography-Mass Spectrometry (GC-MS, Agilent 7890A and Agilent 5975C; Agilent Technologies, Palo Alto, CA, USA) on several occasions. The GC-MS sampling was also applied for the experiments in the environmental chamber but due to some instrumental malfunction, conclusive results have not been obtained from those measurements.

Two types of infrared gas analysers were used for $CO_2$ and $H_2O$ vapour analysis. A LI-7000 non-dispersive infrared gas analyser (Li-COR, Lincoln, Nebraska, USA) was used for the $CO_2$ gas analysis for the experiments performed in the environmental chamber. $H_2O$ vapour analysis has not been performed using this instrument due to lack of calibration data. $H_2O$ vapour was analysed with the help of PTR-MS (see § 3.2.3). For the investigations at DTO, a LI-840A non-dispersive infrared gas analyser (Li-COR, Lincoln, Nebraska, USA) was used for the $CO_2$ and $H_2O$ vapour analysis.

## 2.3 Overview of the dissertation

Chapter 3 contains information about methanol fluxes from maize leaves of three leaf developmental stages: young, semi-mature and mature. Differences in flux intensity and pattern between the different leaf developmental stages, and possible emission drivers are also discussed in this chapter. The data were measured in a light and temperature controlled

environmental chamber over 5 replicates (each) of young, semi-mature, and mature maize leaves. This chapter is based upon the article "Methanol emissions from maize: ontogenetic dependence to varying light conditions and guttation as an additional factor constraining the flux" which has been published in Atmospheric Environment, 2017 (DOI: org/10.1016/j.atmosenv.2016.12.041). The author performed the measurements and data analysis and wrote the manuscript except the part on modelling of methanol emission.

Chapter 4 deals with BVOC fluxes from senescent maize leaves and their comparison with other leaf developmental stages. Compound-specific temporal variability of BVOC emissions and total accumulated BVOC emissions from the senescent leaf are presented. In addition, an estimation of total methanol emissions from a leaf at all leaf developmental stages and from a whole plant is given. This chapter is based on the article "Oxygenated volatile organic compound emissions from senescent maize leaves and comparison with other leaf developmental stages" which has been submitted to Atmospheric Environment and is in review. The author performed the measurements and data analysis and wrote the manuscript except the part on estimation of total methanol emission from a leaf/entire plant.

Chapter 5 provides new information regarding grazing-induced BVOC fluxes from a managed grassland in Belgium. Differences between BVOC fluxes of grazed and undisturbed grassland in terms of intensity and pattern are the main focus of the chapter, which is a draft of a paper "Grazing induced BVOC fluxes from a managed grassland". The author contributed to the measurements and data analysis, and wrote the manuscript.

Chapter 6 summarises the overall conclusions of this dissertation and discusses future perspectives. It brings together all the findings obtained from this work, information which still remains inconclusive, and studies yet to be done.

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# Chapter 3: Methanol emissions from maize: ontogenetic dependence to varying light conditions and guttation as an additional factor constraining the flux

Abstract

Because of its high abundance and long lifetime compared to other volatile organic compounds in the atmosphere, methanol ($CH_3OH$) plays an important role in atmospheric chemistry. Even though agricultural crops are believed to be a large source of methanol, emission inventories from those crop ecosystems are still scarce and little information is available concerning the driving mechanisms for methanol production and emission at different developmental stages of the plants/leaves. This study focuses on methanol emissions from *Zea mays* L. (maize), which is vastly cultivated throughout the world. Flux measurements have been performed on young plants, almost fully grown leaves and fully grown leaves, enclosed in dynamic flow-through enclosures in a temperature and light-controlled environmental chamber. Strong differences in the response of methanol emissions to variations in PPFD (Photosynthetic Photon Flux Density) were noticed between the young plants, almost fully grown and fully grown leaves. Moreover, young maize plants showed strong emission peaks following light/dark transitions, for which guttation can be put forward as a hypothetical pathway. Young plants' average daily methanol fluxes exceeded by a factor of 17 those of almost fully grown and fully grown leaves when expressed per leaf area. Absolute flux values were found to be smaller than those reported in the literature, but in fair agreement with recent ecosystem scale flux measurements above a maize field of the same variety as used in this study. The flux measurements in the current study were used to evaluate the dynamic biogenic volatile organic compound (BVOC) emission model of Niinemets and Reichstein. The modelled and measured fluxes from almost fully grown leaves were found to agree best when a temperature and light dependent methanol production function was applied. However, this production function turned out not to be suitable for modelling the observed emissions from the young plants, indicating that production must be influenced by (an) other parameter(s). This study clearly shows that methanol emission from maize is complex, especially for young plants. Additional studies at different developmental stages of other maize varieties and crop species will be required in order to develop accurate methanol emission algorithms for agricultural crops.

## 3.1 Introduction

Among all atmospheric hydrocarbons, methanol ($CH_3OH$) is the second most abundant volatile organic compound in the troposphere, with mixing ratios ranging up to several tens of parts per billion (Riemer et al., 1998; Singh et al., 2000; Schade and Goldstein, 2001; Jacob et al., 2005, Wohlfahrt et al., 2015). Since it is an important precursor of carbon monoxide, formaldehyde and tropospheric ozone, it plays an important role in the global tropospheric chemistry (Tie et al., 2003; Millet et al., 2006; Duncan et al., 2007; Choi et al., 2010; Hu et al., 2011). Field and laboratory measurements have been carried out to characterize methanol sources and sinks. By integrating this knowledge into global chemistry and transport models, global annual budgets have been constructed (Singh et al., 2000; Heikes et al., 2002; Galbally and Kirstine, 2002; Tie et al., 2003; von Kuhlmann et al., 2003; Jacob et al., 2005; Millet et al., 2008; Stavrakou et al., 2011). Terrestrial plants have been found to be a major source of atmospheric methanol, with an annual global emission ranging from 75 to 280 Tg $y^{-1}$ and constituting 60 to 80 % of the total source strength. Moreover, recent research has revealed bi-directional exchange of methanol between terrestrial ecosystems and the atmosphere. Deposition of methanol is likely to be favoured by the formation of wet layers from which it may be removed chemically or biologically (Wohlfahrt et al., 2015; Laffineur et al., 2012; Niinemets et al., 2014; Seco et al., 2007). In leaves, methanol is mainly produced by the demethylation of pectin (Fall and Benson, 1996). Consequently, changes in cell wall structure related to growth (MacDonald and Fall, 1993; Nemecek-Marshall et al., 1995; Galbally and Kirstine, 2002; Karl et al., 2003; Harley et al., 2007), leaf abscission, the ageing of leaf tissues (Harriman et al., 1991) and intercellular air space generation (Nemecek-Marshall et al., 1995) play an important role in methanol emission from leaves. Therefore, methanol fluxes are affected by the seasonality of the vegetation, i.e. by growth stages and phenological processes (Bracho-Nunez et al., 2011). Several studies already reported that methanol emission from young leaves of various plant species is several times higher than that from mature leaves (MacDonald and Fall, 1993; Nemecek-Marshall et al., 1995; Karl et al., 2003; Custer and Schade, 2007; Harley et al., 2007; Hüve et al., 2007; Bracho-Nunez et al., 2011; Hu et al., 2011; Wells et al., 2012). Furthermore, methanol emission was found to be correlated to stomatal conductance (MacDonald and Fall, 1993; Nemecek-Marshall et al., 1995; Niinemets et al., 2003a), temperature (Schade and Goldstein, 2001; Karl et al., 2003, 2004, 2005; Brunner et al., 2007; Custer and Schade, 2007; Hüve et al., 2007; Folkers et al., 2008) and light conditions (Harley et al., 2007; Hüve et al., 2007; Folkers et al., 2008).

Maize (*Zea mays* L.) was chosen for this study because of its vast cultivation worldwide (13.7 % of the global cropland area, (FAO, 2015)) and because it is a fast-growing crop species which is potentially characterized by large methanol emissions. As methanol emission is the result of Pectin Methyl Esterase (PME) activity (Fall and Benson, 1996), which is in turn dependent on both the rate of cell division and cell expansion (which in turn are under the control of the plant hormones cytokinins (Taiz and Zeiger, 2002)), its emission rate from young developing leaves of fast growing maize plants may be higher than from slower-growing plant species. The little data available in the literature on BVOC emissions from maize (MacDonald and Fall, 1993; Das et al., 2003; Graus et al., 2013) indeed indicate that it could be an important plant species for exchanging methanol with the environment. Those studies, however, only covered a very limited period of the growing season and were conducted in very similar weather conditions. Recently, a field study was conducted to measure methanol exchanges from maize under natural environmental conditions for a whole growing season (Bachy et al., 2016). These flux measurements were performed at ecosystem-scale using the eddy covariance technique, thereby encompassing both soil and plant exchanges. Consequently, knowledge about methanol exchanges by the maize plant itself and their underlying exchange mechanisms remains limited. The present study aims to fill this knowledge gap by 1) evaluating the impact of varying PPFD on methanol emissions at constant temperature conditions in the environmental chamber, 2) studying the effect of leaf age on the methanol emission pattern and magnitude and 3) by confronting our measurements with the dynamic BVOC emission model of Niinemets and Reichstein (Niinemets and Reichstein, 2003a and 2003b) using different methanol production functions.

## 3.2 Materials and methods

### 3.2.1 Plants and environmental conditions

Investigations were carried out on silage maize (*Zea mays* L., variety Prosil, Caussade Semences, France) at three different life stages: young, middle age and fully grown (5 replicates for each stage). In what follows, these stages will be referred to as stage 1, stage 2 and stage 3, respectively. At stage 1, measurements were carried out on plants from 4 up to 14 days old (age counting began with seed germination). Four-day-old plants were about 10 cm tall and had 2 small leaves (leaf numbering started from the base). Fourteen-day-old plants were about 35 cm tall and had 4 to 5 leaves. The whole plant was enclosed at this stage because it was not feasible to enclose a single leaf for a sufficiently long period without

damaging it. This was due to the fast elongation rate of both leaves and stem. An almost fully grown 7th leaf (total length was about 80 cm) of a 30 to 40-day-old plant (about 120 cm tall) was partially enclosed (the top 55 cm) during the experiments on leaves of stage 2. At stage 3, a fully grown leaf (either the 7th, 8th or 9th) of a fully grown maize plant (about 180 cm tall) was partially enclosed (the top 55 cm as well). After enclosing, the measurements on leaves of stage 2 and 3 were performed for about 5 days. More details about the plants and the enclosed leaf/leaves at the different plant developmental stages at which the experiments were carried out can be found in Table 3.1.

Table 3.1: Detailed information about the plants and the enclosed leaf/leaves at the different plant developmental stages at which the experiments were carried out. The BBCH code and AGDD (Accumulated Growing Degree-Days), two useful metrics for describing plant development, are explained in supplement S1.

| Stage | Plant age [days] | Enclosed leaf/leaves | BBCH code | AGDD [degree-days] |
|---|---|---|---|---|
| 1 | 4-14 | first 2-5 leaves (young) | 10-14 | 122-274 |
| 2 | 30-40 | 7th leaf (almost fully grown) | 17-19 | 518-671 |
| 3 | 60-70 | 7th/8th/9th leaf (fully grown) | 65-69 | 976-1128 |

When the seeds had germinated, the small seedlings were transplanted in cylindrical 20 L pots containing soil that consisted of a mixture of 75 % silty clay loam and 25 % sand (volume/volume). Plants were grown in the environmental chamber where the BVOC measurements were conducted. They were watered regularly to keep the soil moisture content around 35%.

The dimensions of the environmental chamber were 3 m x 2.6 m x 2.2 m (L x W x H). Light intensity and temperature were controlled automatically. Seven-hour-long dark periods were alternated by photoperiods of seventeen hours. Light was provided by a set of 40 Philips Green Power Production LED Modules (Lights Interaction Agro B.V., Eindhoven, The Netherlands, model # R/W 120) mounted at the environmental chamber ceiling. The LED panels provided light mainly at the blue and red light wavelength region of the spectrum.

PPFD in the environmental chamber was varied stepwise, resulting in 6 light periods ranging from 0 to 600 µmol $m^{-2}$ $s^{-1}$ at the top of the plant enclosures (Figures 3.2a, d and g). During the photoperiod, the temperature in the growth chamber was kept at 25 °C by an air cooling system. In the absence of light, temperature slowly decreased to around 23 °C as no additional heating was provided. Temperature in the enclosures varied depending on the light conditions from 23 to 27 °C.

### 3.2.2 Dynamic flow-through enclosures

Cylindrical dynamic flow-through enclosures were used for enclosing a whole maize plant or part of a single leaf (Figure 3.1). The frame was made of aluminum bars and transparent polymethylmethacrylate (PMMA) rings and plates. It held a cylindrical 50 µm thick PFA envelope (Norton, Saint-Gobain Performance Plastics, NJ, USA) which was 96 % transparent to photosynthetically active radiation (PAR). Two ¼ inch outer diameter PFA tubes were connected to each enclosure, one for supplying purge air (with flow rate $Q_{in}$) and the other for sampling BVOC-enriched air (with flow rate $Q_{out}$). The enclosures were equipped with a Teflon fan for efficient mixing of emitted BVOCs and purge air, a thermistor (type 10k, NTC, Omega, UK) and a relative humidity sensor (type HIH-3610, Honeywell, NJ, USA ) for continuously monitoring air temperature and relative humidity, respectively. Besides, a PAR sensor (type LI-190SA, LI-COR, USA) was fixed in the middle of the environmental chamber at the top height of the enclosures to track PPFD values on the plants or enclosed leaves.

Two enclosure configurations were used, depending on the enclosed biomass. Small shoots of plants of stage 1 were inserted in 22 L enclosures through a small opening (1 cm diameter) in the bottom foil of the enclosure and were left to grow inside until they touched the upper foil (Figure 3.1, left). In this way emissions from shoots could be separated from emissions from roots and soil. In the second configuration, the same type of enclosure was fixed to a mast and the lower foil was replaced by an extension of the cylindrical envelope which was gently put around a leaf of stage 2 or stage 3 (enclosing the top 55 cm of the leaf) (Figure 3.1, right). In this way, the total enclosure volume was enlarged to about 30 L. The foil around the enclosed leaf was kept slightly loose to prevent mechanical damage of the leaf. In addition, an empty enclosure was used as a reference to measure the background VOC concentration.

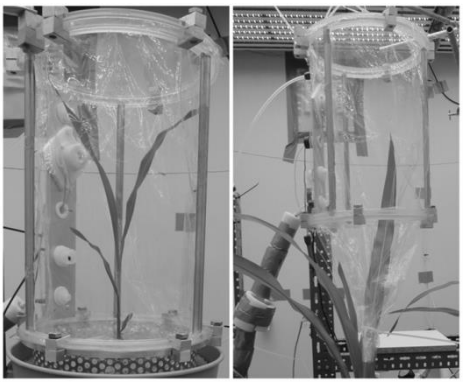

Figure 3.1: Dynamic enclosure set-up for fully enclosed young maize plants (left) and for partially enclosed almost fully grown maize leaves (right).

### 3.2.3 Gas exchange measurements, VOC sampling and quantification

An oil free compressor with absorption dryer (DK50 2x2V/110 S/M/230V, EKOM, Germany) was used to provide air to the enclosures. Since air from the compressor had a much lower $CO_2$ concentration (approx. 200 ppmv) than the ambient air, pure $CO_2$ from a commercial gas bottle (Air products, 99.5 % pure) was added to the air flow by means of a flow controller (MKS Instruments, Andover, MA, USA). An average concentration of 420 ppmv $CO_2$ was maintained in the purge air flow all the time. Relative humidity was adjusted to about 40 % by a homemade humidifier, as the relative humidity of the compressed/dried air was very low (about 5 %). Air was subsequently sent through a set of active charcoal filters (Airpel 10, Organosorb 10-CO, Desotec, Belgium) to scrub VOCs from the humidified air. To obtain equal flow rates for all the enclosures, the filtered air was then sent through a set of flow meters (F-102EI-ABD-55V, 0-30 L $min^{-1}$, Bronkhorst, The Netherlands), each followed by a ball valve (type SS-43S4, Swagelok, OH, USA) for manual adjustment of the flow rate($Q_{in}$) which was maintained at 5 L $min^{-1}$ (at standard conditions of pressure (1013.25 hPa) and temperature (293 K)) for all individual enclosures. $Q_{in}$ was always much higher than $Q_{out}$ (kept at 1.35 L $min^{-1}$) and the overflow ($Q_{in}$ - $Q_{out}$) left the enclosure mainly through the open space around the enclosed stem or leaf. The pressure in the enclosures was less than 0.2 hPa above ambient pressure.

BVOC mixing ratios were measured using a conventional quadrupole-based hs-PTR-MS (Proton Transfer Reaction–Mass Spectrometer, Ionicon Analytik G.m.b.H., Innsbruck, Austria), which is a very sensitive instrument for real time monitoring of trace volatile

organic compounds (Lindinger et al, 1998; Ellis and Mayhew, 2014). The PTR-MS was operated at a drift tube pressure of 2.1 hPa, a drift tube temperature of 60 °C and a drift tube voltage of 600 V, resulting in a value for E/N (ratio of the electric field E to the gas number density N) in the drift tube of 130 Td (1 Td = $10^{-17}$ V $cm^2$). Furthermore, the inlet line of the PTR-MS was kept at 60 °C. Ions at m/z 33 and isotopes of the proton hydrates at m/z 21 ($H_3{}^{18}O^+$) and m/z 39 ($H_5{}^{16}O{}^{18}O^+$) as well as some other BVOC related ion species were followed sequentially. The ion signal at m/z 33 is known to be due to both protonated methanol ($H^+.CH_3OH$) and the molecular oxygen isotope $^{17}O^{16}O^+$. The contribution of the latter ion signal, however, cancels when determining the net ion signals (plant/leaf enclosure – reference enclosure) for calculating the methanol fluxes. The ion signal at m/z 39, which is used here to calculate water vapor fluxes, could in principle contain contributions of fragment ions from BVOCs as well. However, net ion signals at m/z 39 were always significantly higher than net VOC-related ion signals at other m/z values (except for m/z 33), which suggests that the contribution of VOCs to m/z 39 in our study must have been very small.

The total PTR-MS cycle time was 44 s and during this time, the ion signal at m/z 33 was measured for 10 s. Additionally, PTR-MS calibrations were performed every 5 days by using a gravimetrically prepared mixture of methanol (1.07 ppmv) and other BVOCs in nitrogen (Apel-Riemer Inc., Denver, CO, USA), with a certified accuracy of 5 %. The calibration gas was further diluted with zero air, generated by sending ambient air through a catalytic converter (Parker® ChromGas® Zero Air Generator, model 1001, Parker Hannifin Corporation, Haverhill, MA, USA) to obtain mixing ratios in the 0-12 ppbv range for methanol. The methanol detection limit was estimated at 170 pptv (for an integration time of 10 seconds).

The calibration of the water vapor mixing ratio in the sampled air versus the m/z 39 ion signal was performed on average every 5 days by using a dew point generator (LI-COR 610, LI-COR, Lincoln, Nebraska, USA). Besides, carbon dioxide ($CO_2$) mixing ratios were determined with a LI-7000 non-dispersive infrared gas analyzer (LI-COR, Lincoln, Nebraska, USA).

In order to perform flux measurements with sufficient time resolution, the number of sampling enclosures was limited to three: two plant or leaf enclosures and one empty reference enclosure. Consequently, not all plant replicates were investigated simultaneously, but they were studied under the same experimental conditions. The diurnal cycle of BVOC

emissions was investigated by sequentially sampling air from each enclosure for a duration of 10 minutes, resulting in a measurement cycle time of 30 minutes. This sampling procedure was automated by a Labview™ (National Instruments Corporation, Austin, Texas, USA) controlled manifold system. The first and the last minute of the 10-minute sampling period were not considered for the flux calculations to avoid measurement errors due to switches between enclosures. Measurements performed at the day of enclosure were not taken into account as they might have been influenced by stress-induced emissions. Methanol emission rates ($E_{CH3OH}$), transpiration rates (Tr) and net photosynthesis rates (P), all expressed in mol $m^{-2}$ $s^{-1}$, were obtained by using the following equations:

$$E_{CH3OH} = \left(X^{air}_{CH3OH,PE} - X^{air}_{CH3OH,RE}\right) \times \frac{Q_{in}}{RT_{ref}} \times \frac{1}{LA} \quad (3.1a)$$

$$Tr = \left(X^{air}_{H2O,PE} - X^{air}_{H2O,RE}\right) \times \frac{Q_{in}}{RT_{ref}} \times \frac{1}{LA} \quad (3.1b)$$

$$P = \left(X^{air}_{CO2,PE} - X^{air}_{CO2,RE}\right) \times \frac{Q_{in}}{RT_{ref}} \times \frac{1}{LA} \quad (3.1c)$$

In these equations $X_{A,PE}^{air}$ and $X_{A,RE}^{air}$ are the mole fractions of compound A (either $CH_3OH$, $H_2O$ or $CO_2$) in the sampled air flows from the plant/leaf and reference enclosures, respectively. $Q_{in}$ [Pa $m^3$ $s^{-1}$] and $T_{ref}$ (293 K) are the incoming flow rate (measured with the mass flow meter) and the reference temperature of the mass flow meter, respectively. R is the ideal gas constant (8.314 J $mol^{-1}$ $K^{-1}$) and LA [$m^2$] is the enclosed leaf area.

The total leaf conductance $G_{tot}$ [mol $m^{-2}$ $s^{-1}$] to water vapor was estimated by the following formula (Pearcy et al., 1989):

$$G_{tot} = \frac{Tr}{(X^{leaf}_{H2O} - X^{air}_{H2O,PE})} \quad (3.2)$$

in which $X_{H2O}^{leaf}$ is the water vapor mixing ratios in the leaf's intercellular spaces. This mixing ratio is given by Eq. 3.3, in which $p_{sat,Tleaf}$ [Pa] is the saturation vapor pressures of water at $T_{leaf}$.

$$X^{leaf}_{H2O} = \frac{p_{sat,Tleaf}}{101325} \quad (3.3)$$

As the position of the leaves in the enclosures continuously changed due to the fast growth rate of the young plants, leaf temperature measurements with IR leaf temperature sensors or a

thermocouple were experimentally not feasible. Therefore leaf temperature was assumed to be equal to air temperature. The leaf conductance $G_{tot}$ in Eq. 3.2 includes stomatal conductance, boundary layer conductance and cuticular conductance. In our set-up, however, boundary layer conductance and the difference between air and leaf temperature were assumed to be limited by the purge air flow, as well as by the efficient mixing of the air in the enclosure by the Teflon fan. By neglecting cuticular conductance, stomatal conductance, $G_S$, in our experiments was therefore approximated by total leaf conductance.

### 3.2.4 Leaf area estimation

Due to the fast growth of maize plants of stage 1, daily leaf area estimations were needed. Since opening the enclosure could have damaged the plants and could have induced stress related BVOC emissions, they were kept enclosed during the entire 10-day measurement period. During this period, leaf area was determined daily by visual estimation of the maximum width W and length L of the individual leaves without opening the enclosure. The leaf area was estimated as the product of W and L, multiplied by a factor 0.78. This factor was experimentally determined using all leaves of a set of 5 non-enclosed plants which were grown in similar conditions as the enclosed ones and is in good agreement with the literature (Mokhtarpour et al., 2010). At the end of the measurement period, the total leaf area was measured accurately (Harley et al., 2007) after the removal of all the leaves from the plant. The data obtained agreed well with the visually estimated leaf dimensions just before disclosing the plant. Fresh weight of the leaves was measured immediately after disclosure and their dry weight was obtained after at least 48 hours of drying in an oven at 75 °C until all water was evaporated and a constant weight was reached.

Leaf area estimations of partially enclosed leaves of stage 2 and stage 3 were also performed on a daily basis. Accurate leaf area and fresh weight measurements were also performed immediately after removing the leaves from the enclosures. For the dry weight measurements, the same procedure was followed as for plants of stage 1.

### 3.2.5 Methanol emission modelling

There is strong evidence that leaf-level emissions of highly water-soluble species, such as methanol, are controlled by stomatal conductance (Harley et al. 2013). Consequently, those emissions can only be properly described by a model that predicts the response of the emissions to variations in stomatal conductance, such as the one of Niinemets and Reichstein (Niinemets and Reichstein, 2003a and 2003b). Therefore, flux data for the leaves of stage 2

and 3 and maize plants of stage 1 have been evaluated against the dynamic BVOC emission model of Niinemets and Reichstein, using a pre-defined methanol production function.

The experimental data for stomatal conductance, temperature and PPFD were used as input for the model. By lack of specific data for *Zea Mays* L., the leaf structural parameters for *Phaseolus vulgaris* L. as reported in (Niinemets and Reichstein, 2003b) were used instead. In accordance with Niinemets and Reichstein (2003b), our model results were not strongly affected by changes in those leaf structural parameters.

Although some studies indicate that methanol production in leaves is independent of PPFD (Oikawa et al., 2011; Harley et al., 2007) and attribute the PPFD dependence of the emissions to the effect of PPFD on stomatal conductance, we considered a more general approach by using a similar light and temperature dependence for the methanol production function as in the leaf level methanol emission algorithm in the MEGANv2.1 model (Stavrakou et al., 2011). This methanol production function PF is given by:

$$PF = \varepsilon \cdot \gamma_{PT} \tag{3.4}$$

where ε is the rate of methanol production at the standard leaf temperature and PPFD conditions of 303 K and 1000 µmol $m^{-2}$ $s^{-1}$, respectively. $\gamma_{PT}$, the leaf level temperature and light response factor, is given by:

$$\gamma_{PT} = (1 - LDF) \cdot \gamma_{T-li} + LDF \cdot \gamma_P \cdot \gamma_{T-ld} \tag{3.5}$$

LDF is the light dependent fraction of the emissions, $\gamma_P$ is the light dependent response factor and $\gamma_{T\text{-}ld}$ and $\gamma_{T\text{-}li}$ are the temperature response factors of the light-dependent and the light-independent methanol emissions, respectively. The explicit formulas for these response factors can be found in Stavrakou et al., 2011. A value of 0.08 $K^{-1}$ was used for the β parameter in the exponential temperature dependence of $\gamma_{T\text{-}li}$ (Stavrakou et al., 2011; Harley et al., 2007). More details about the equations used for modelling methanol fluxes and model options are given in Supplementary section S3.1.

## 3.3 Results and discussion

### 3.3.1 Daytime methanol production driver(s) for young maize plants

The daytime methanol emission pattern for the maize plants of stage 1 was characterized by a steadily increasing emission for most of the day, upon which transient emissions were superimposed after each increase in PPFD (Figure 3.2a-c). According to the dynamic BVOC emission model of Niinemets and Reichstein (Niinemets and Reichstein, 2003a and 2003b),

these transient changes are due to a transfer of methanol from the non-specific liquid storage pool in the leaves to the gaseous pool, following a sudden increase in stomatal conductance. The transient changes last until a new equilibrium between the pools is settled. The first transient increase of methanol emission, along with a stepwise increase of Gs, Tr and P, was observed after the plants had been exposed to a PPFD of approximately 100 µmol $m^{-2}$ $s^{-1}$ (at 5 a.m.) following a dark period of 7 hours. Similar bursts in the morning have been reported previously by many investigators (MacDonald and Fall, 1993; Nemecek-Marshall et al., 1995; Hüve et al., 2007; Harley et al., 2007; Folkers et al., 2008). Small transient peaks of methanol emission were also noticed at every further stepwise increase in PPFD (at 8 a.m. and 11 a.m.). This has also been observed in laboratory studies of *Sorghum bicolor* and *Pinus taeda* (Harley et al., 2007) and is related to accompanying stepwise increases in stomatal conductance.

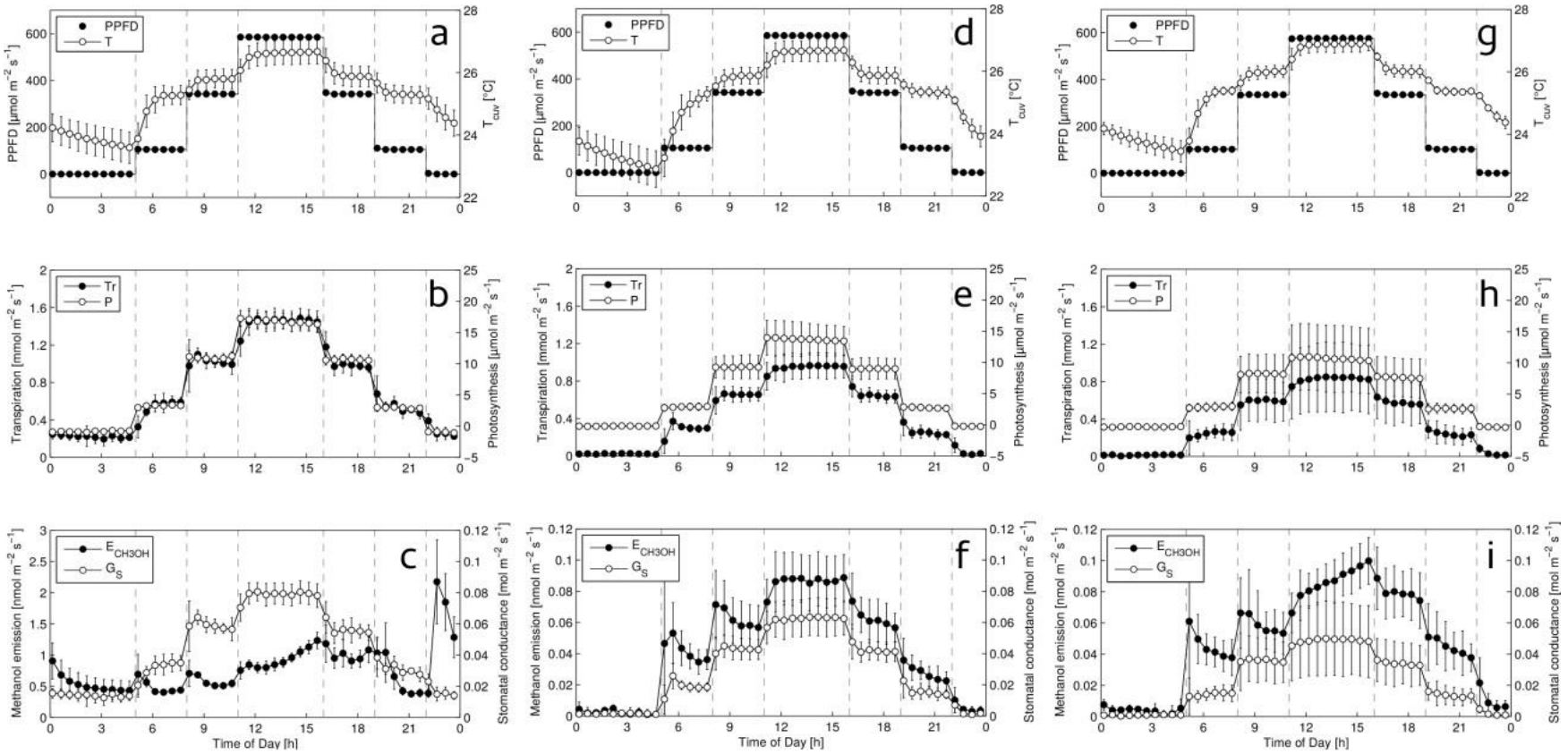


Figure 3.2: Average PPFD, temperature, transpiration, photosynthesis, methanol emission and stomatal conductance for the maize plants of stage 1 (a-c) and leaves of stage 2 (d-f) and stage 3 (g-i) subjected to a symmetric PPFD pattern. Error bars correspond to standard deviations. Averaging was performed over 5 replicates and over the last 6 days (day 9 to 14) of the measurement period

for the plants of stage 1 and over 5 replicates and periods of 3 days for leaves of stage 2 and stage 3. Vertical dashed grey lines indicate the time of the stepwise PPFD increases/decreases.

Although a symmetric light pattern was maintained in the environmental chamber, a strong asymmetry was observed in the methanol emission pattern (Figure 3.2a and 3.2c). Indeed, for all constant PPFD periods between 5 a.m. and 4 p.m., the transient methanol emission peaks were followed by gradual increases in methanol emission. Moreover, the rate of increase of the emissions became larger with time. The decrease in PPFD at 4 p.m. resulted in a decrease in methanol emissions, which was again followed by a slightly upward trend during this constant PPFD period. Consequently, the average methanol emission at 350 µmol $m^{-2}$ $s^{-1}$ in the afternoon (approximately 1.0 nmol $m^{-2}$ $s^{-1}$) was about twice as large as the emission at the same PPFD level before noon (approximately 0.5 nmol $m^{-2}$ $s^{-1}$, not considering the transient emission values at the start of this constant PPFD period). This upward trend could be explained by an increased methanol production over the day, possibly related to the diurnal evolution of plant growth rate (which is known to be species dependent (Hüve et al., 2007)) or to a diurnal variability in PME activity. A decrease in methanol emission was also noticed after the next decrease in PPFD (at 7 p.m.), albeit with some delay. But now the emissions showed a downward trend and finally reached a value close to the one at the end of the dark period (5 a.m.).

The continuous increase in methanol emission during the period of maximal PPFD suggests that the methanol production has not yet arrived at a maximum before the decrease in PPFD at 4 p.m. (Figure 3.2c). To find out for how long this rise in methanol emission would continue at constant maximal PPFD conditions, a new experiment was performed with similar plant material in which the period of maximal PPFD was prolonged by 6 hours (up to 10 p.m.), after which PPFD dropped to zero. The results of this experiment are shown in Figure 3.3. Whereas the methanol emission rate in both experiments was very similar within the photoperiod between 5 a.m. and 4 p.m., it continued to increase smoothly in the experiment with the extended period of maximum PPDF (Figure 3.3c), peaked around 7 p.m. and then decreased towards the end of the maximum PPFD period. Transpiration, photosynthesis and stomatal conductance hardly varied during this entire period of maximum PPFD. The variation of methanol emission during this period was thus clearly not controlled

by the stomata, but probably by changes in the methanol production rate. These changes are possibly induced by a temporal variability in growth rate or PME activity.

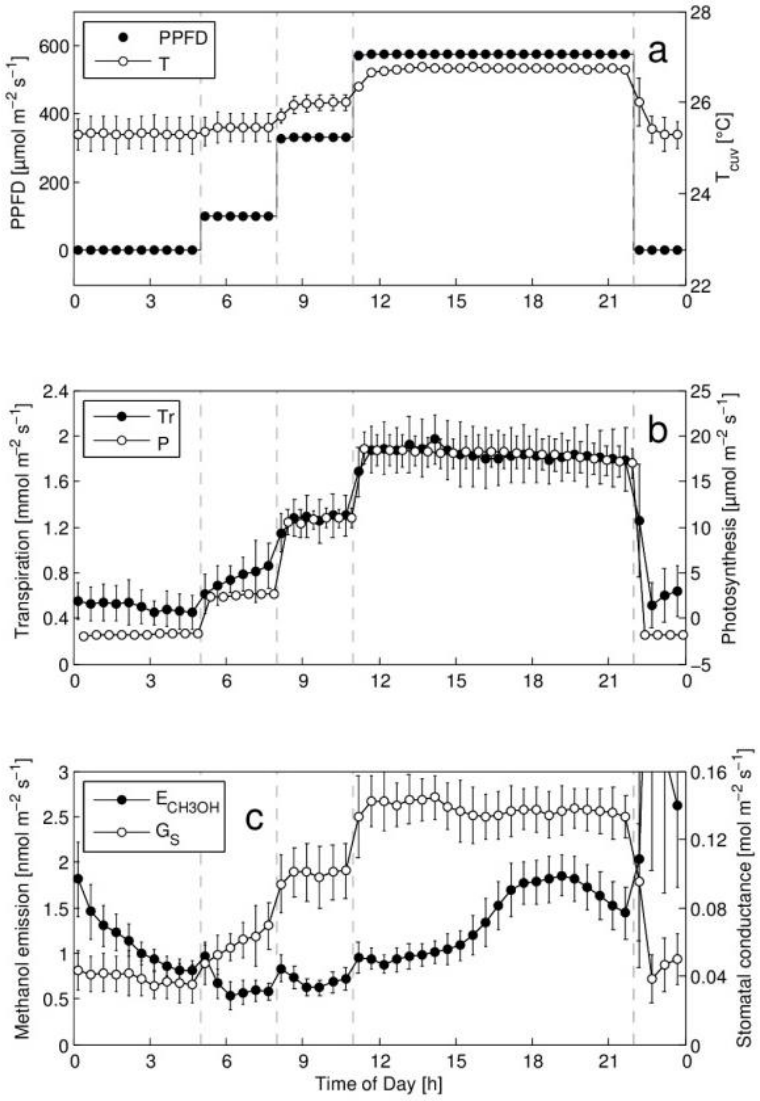


Figure 3.3: Average PPFD, temperature, transpiration, photosynthesis, methanol emission and stomatal conductance for the maize plants of stage 1 subjected to an asymmetric PPFD pattern with 11 hours of maximum PPFD. Error bars correspond to standard deviations. The data were averaged over 2 replicates and over the last 6 days (day 9 to 14) of the measurement period. Vertical dashed grey lines indicate the time of the stepwise PPFD increases/decreases.

### 3.3.2 Is methanol production for almost fully grown and fully grown maize leaves controlled by PPFD?

Maize leaves of stage 2 and stage 3 showed stepwise changes of methanol emission following stepwise changes in PPFD (Figures 3.2d, 3.2f, 3.2g and 3.2i). Whereas the methanol emissions followed very closely transpiration and stomatal conductance for the leaves of stage 2, a gradual increase in the emissions during the maximum PPFD period was noticed for the leaves of stage 3. This results in an asymmetric emission pattern under a symmetric PPFD pattern. Small methanol emission bursts, resulting from changes in stomatal conductance, were superimposed upon the stepwise increase in emission for leaves of both stages 2 and 3 when PPFD increased (at 5 a.m. and 8 a.m.).

Recent studies by Oikawa et al. on *Lycopersicon esculentum* (Oikawa et al., 2011) indicate that methanol production from that plant species is independent of PPFD, and variation of the methanol emissions with PPFD is related to the impact of this parameter on stomatal conductance. According to the model of Niinemets and Reichstein, however, changes in stomatal conductance at constant methanol production should only lead to transient methanol emission changes and not to stepwise increases or decreases. Possible explanations for the stepwise changes in methanol emission with PPFD for the leaves of stages 2 and 3 could be 1) that methanol production is actually controlled by PPFD for those leaves and/or 2) that methanol mainly comes with the transpiration stream which carries methanol from other parts of the plant (roots, stem, fruits, flowers …) to the leaves.

In order to find out whether a PPFD dependence of the methanol production could explain our observations, we applied the Niinemets and Reichstein model using the generalized production function described in Section 3.2.5 (Eq. 3.4). In a study on leaf-level methanol emissions from different plant species, Harley et al. (2007) found a nice correlation between modelled and measured methanol data by using a production function that varied exponentially with temperature. This production function is obtained by setting the light dependent fraction of the emission (LDF in Eq. 3.5) to zero. We have used it in a first step to evaluate our measurements for the maize leaves of stage 2 against the model. A standard methanol production rate ε of $6.3 \times 10^{-11}$ mol $m^{-2}$ $s^{-1}$ was obtained by minimizing the absolute difference between the accumulated measured and modelled emissions over the whole day. The model result is shown in Figure 3.4 (full gray line) together with the measurements. The model largely overestimates the emission burst following the dark/light transition and the

nighttime emissions preceding this burst. Moreover, the variation of the methanol emissions with PPFD at equilibrium conditions is not well reproduced either. Harley et al. (2007) reported that any change to the model that retards the rate at which the liquid methanol pool comes into equilibration improved the model fit to their data. One way to extend this time for equilibration in the model is by increasing the value for the liquid water content (LWC) of the leaves. They found a better agreement between their modelled and observed transient methanol emissions by multiplying this LWC value by a factor 4. By using the same multiplication factor, the emission bursts in our experiments were also better simulated by the model. Nevertheless, the modelled daily emission pattern (dashed gray line) still agreed poorly with the measurements. Moreover, as Harley et al. (2007) pointed out in their paper, this way of extending the pool equilibration time cannot be justified as it leads to unrealistically high values for the liquid water content of the leaves.

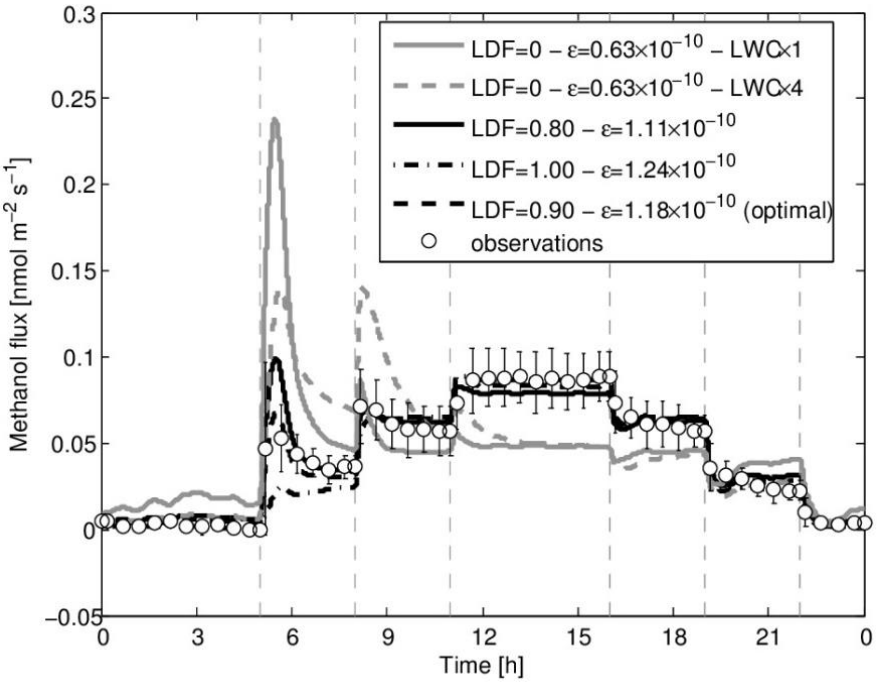


Figure 3.4: Observed (individual data points) and modelled (lines) methanol emissions for the leaves of stage 2. Vertical dashed grey lines indicate the time of the stepwise PPFD increases/decreases. LDF is dimensionless and ε is expressed in mol $m^{-2}$ $s^{-1}$. See text for further details.

Subsequently, a light and temperature dependent production function was applied and in a first stage LDF was kept at the prescribed value of 0.8 as in the MEGANv2.1 model. An optimal value for ε of $1.11 \times 10^{-10}$ mol $m^{-2}$ $s^{-1}$ was obtained by a least squares fit of the model

to the half hourly emission measurements (Figure 3.4, solid black line). Even though the modelled emissions show a clear improvement with respect to those obtained by using the temperature-only production function, nighttime emissions and the morning peak are still too high compared to the measurements. This is due to an overestimation of the light-independent production (i.e. an underestimation of the LDF value), which results in a too high value of the methanol liquid pool size before the dark/light transition. An overnight light-independent methanol production is, however, necessary to obtain a sufficiently high liquid pool size to be able to simulate the peak in the morning. This is clearly shown in Figure 3.4 (dot-dashed black line) for the model run with LDF=1 (optimized ε is $1.24 \times 10^{-10}$ mol $m^{-2}$ $s^{-1}$).

Therefore, a logical step was to run the model over a large range of both ε and LDF. The least squares fit of the model to the half hourly measurements resulted in values of ε and LDF of $1.18 \times 10^{-10}$ mol $m^{-2}$ $s^{-1}$ and 0.90, respectively (Figure 3.4, dashed black line). Although the modelled emissions for these optimized ε and LDF values show a fair agreement with the observations, some minor observed features such as the small emission burst following the PPFD increase at 8 a.m. and the slow decrease of the emissions after every decrease in PPFD, still cannot be well reproduced. In conclusion, the results clearly indicate that the addition of a PPFD dependence of the methanol production function was definitely required to explain the observed fluxes.

A least squares fit of the model to the half hourly emission measurements of leaves of stage 3 resulted in optimal values of $1.28 \times 10^{-10}$ mol $m^{-2}$ $s^{-1}$ and 0.90 for ε and LDF, respectively (data not shown). This LDF value is the same as the one obtained for the leaves of stage 2. Although the modelled emissions still lie within the error bars of the observations, the agreement is less good than for the leaves of stage 2. This is mainly due to the steady increase of the emissions at a maximum PPFD (Figure 3.2i). The reason for this is unclear, but might be related to cell degradation as the leaves of stage 3 were close to undergoing chlorosis/senescence, a plant developmental stage which is accompanied by increased methanol emissions.

Fitting the model to the methanol emissions from maize plants of stage 1 did not result at all in reasonable predictions of the measurements (Figure 3.5). Indeed, whereas the model results in nice stepwise emission changes during the day, upon which transient effects due to sudden stomatal changes are superimposed, we clearly observed strong variations in emissions at constant light, temperature and stomatal conductance. It is clear that in order to

obtain a better agreement between modelled and measured emissions, the production of methanol from leaves of stage 1 must be modulated by some other parameter (e.g. diurnal variability in leaf growth rate or in PME enzyme activity). We were not able to identify this parameter on the basis of our measurements.

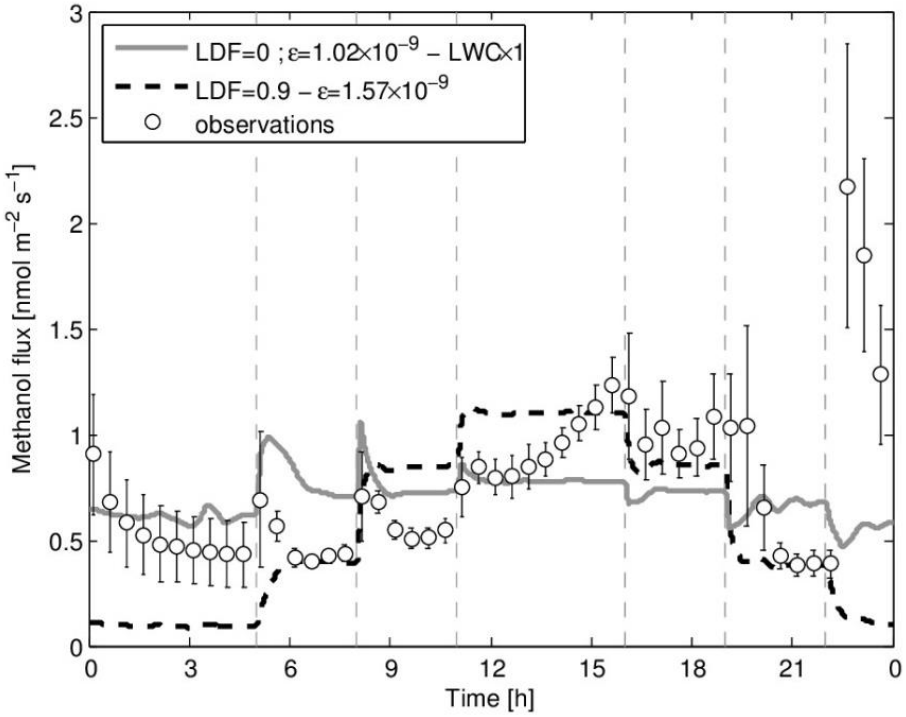


Figure 3.5: Observed (individual data points) and modelled (lines) methanol emissions for the plants of stage 1. The LDF value of 0.9 corresponds to the optimal value found for mature leaves of stage 2 (see Fig. 3.4). LDF=0 corresponds to a light-independent production function. The fit assumes equal accumulated, modelled and measured emissions between 5 a.m. and 10 p.m.. Vertical dashed grey lines indicate the time of the stepwise PPFD increases/decreases. LDF is dimensionless and ε is expressed in mol $m^{-2}$ $s^{-1}$.

### 3.3.3 Guttation as a potential source of nighttime methanol emission from young maize plants

The most striking feature of the methanol emission pattern from maize plants of stage 1 (Figure 3.2c) was the presence of a large peak following the light/dark transition at 10 p.m., which, to the best of our knowledge, has never been mentioned in the literature. In this paper, we put forward the hypothesis that the evaporation of methanol from guttation fluid, coming out of the tips and edges of the plant's young leaves through hydathodes, is a possible cause

for this nighttime peak. Guttation is a common process for young maize plants (Joachimsmeier et al., 2011) and occurs when transpiration reduces after stomatal closure in dark conditions (Singh, 2013). Shortly after turning off the lights in the growth chamber, guttation droplets were indeed found to form on maize leaves of stage 1 on several occasions.

In order to strengthen our hypothesis of guttation as a pathway for methanol emission, guttation droplets from non-enclosed maize plants in the environmental chamber were collected with a Teflon syringe (Torvic, 20 mL) and injected in a similar enclosure as the reference enclosure described in Section 3.2.2. The only difference was that a perforation of 4 mm in diameter was made in the top foil of the enclosure to allow the injection of the fluid. Moreover, purge flow, temperature and PPFD conditions were similar as for the plant emission measurements. Methanol was indeed found to be present in the guttation fluid. The cumulative mass of methanol exiting the guttation fluid, normalized with respect to the total mass of the injected guttation fluid, is shown as a function of time after injection in Figure 3.6.

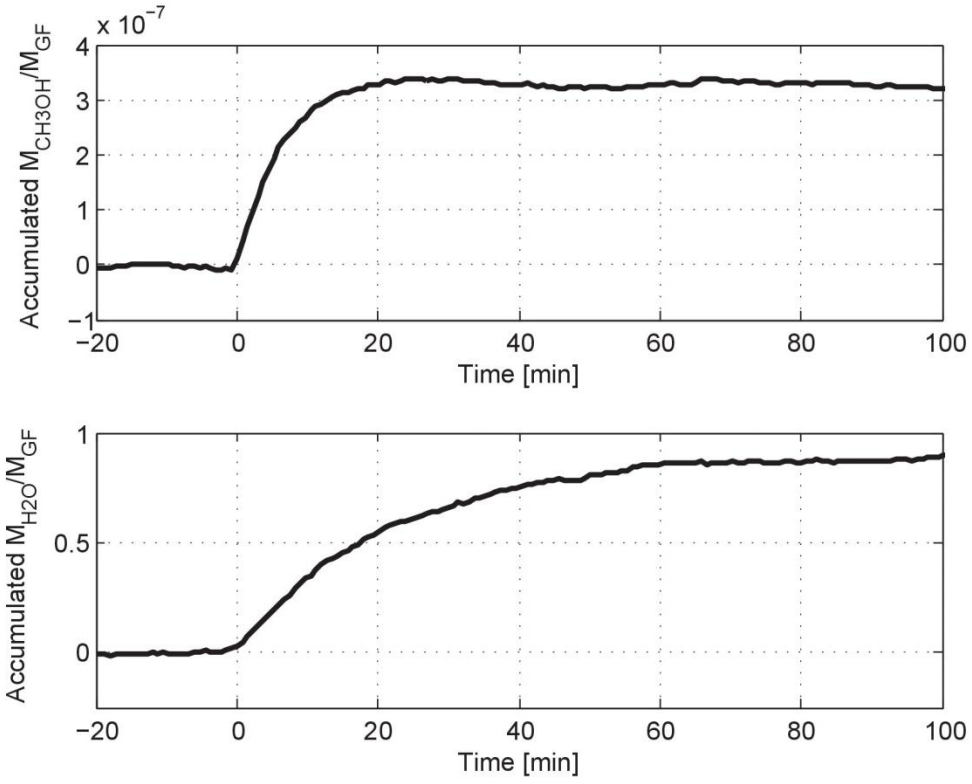


Figure 3.6: Time evolution of the cumulative mass of methanol $M_{CH3OH}$ (upper figure) and water $M_{H2O}$ (lower figure) exiting the guttation fluid, normalized with respect to the

total mass of the injected guttation fluid $M_{GF}$, after injection of the guttation fluid in the enclosure.

The mass mixing ratio of methanol in the guttation fluid was found to be $(3.3 \pm 1.1) \times 10^{-7}$ (n=5). The cumulative mass of evaporated water, again normalized with respect to the total mass of the injected guttation fluid, is also shown in Figure 3.6. Note that the water mass mixing ratio in the guttation fluid is close to 1, indicating that the latter is mainly composed of water. Furthermore, methanol inside the injected liquid completely vaporized within 20 minutes, whereas it took water (from the injected droplets) almost an hour. This difference in temporal evolution of the cumulative mass mixing ratio of water and methanol reflects the difference in volatility between the two compounds but may also be influenced by other factors such as the concentrations of methanol and water vapor in the purge air flow, and temperature and turbulence in the enclosure. The difference in volatility between methanol and water also suggests that part of the methanol in the guttation droplets may already have escaped the liquid phase before the droplets were collected, since it took some time (about 1-1.5 hours) for collectable droplets to form and for the involved scientists to collect a sufficient number of them (approximately 0.5 g of guttation fluid). Consequently, the experimentally obtained value for the methanol mass mixing ratio in the guttation fluid could be considered as a lower limit. This might explain why the large increase in methanol emission following light/dark transition in Figure 3.2c is not accompanied by a noticeable increase in the flow of water vapor (Figure 3.2b). During the experiments with young maize plants subjected to an asymmetric PPFD pattern (Figure 3.3), however, a small increase in the emission of water vapor was noticed after the light/dark transition. This is probably due to a more intense guttation as a result of the larger decrease in PPFD, which also explains the higher nighttime peak value for methanol emissions (Figure 3.3c).

In order to find out whether methanol was also present in the guttation fluid of other crop species than maize, similar experiments were carried out with winter wheat (*Triticum aestivum* L.). It turned out that young winter wheat plants also showed strong guttation and enhanced methanol emissions following light/dark transitions (data not shown). The methanol content of the guttation fluid of 2 sets of 30 young wheat plants (5 to 6 days after germination) was determined in a similar way as for maize. The experiment resulted in values of $2.5 \times 10^{-7}$ and $4.3 \times 10^{-7}$ for the mass mixing ratio of methanol in the guttation fluid, which lie well within the range of values obtained for maize. Further research on other plant species is

definitely required in order to find out whether methanol emission by the guttation pathway is a general mechanism in nature.

The intensity of the large nighttime methanol emission peak appeared to be maximal at the beginning of the six-day period over which the data were averaged (from day 9 to day 14 with respect to seed germination). It then decreased quasi linearly at an average rate of 12 % per day, as reflected by the large error bar on the averaged peak values (Figure 3.2c). This decrease of guttation intensity with time is in line with previous observations on strawberry leaves (*Fragaria x ananassa* Duch.). These showed that, with time, the water pores in the hydathodes get occluded by shield-like plates, which are presumably comprised of epicuticular waxes and substances secreted through the hydathodes (Takeda et al., 1991). Moreover, field studies on agricultural crop species have shown that guttation frequency is particularly high in the early growth stages of the crops (Joachimsmeier et al., 2011).

For plants up to 8 days old (and even 10 days for one of the replicates), a transient methanol peak also occurred after the light intensity decreased from 350 to 100 µmol $m^{-2}$ $s^{-1}$ (data not shown). The hypothetical assignment of the latter peak to the guttation phenomenon is endorsed by the occasional observation of guttation droplets when maize plants of stage 1 were suddenly subjected to lower non-zero PPFD levels.

Beside guttation, other mechanisms for the nighttime methanol emission from plants of stage 1 cannot a priori be excluded. Since methanol emission has been associated with leaf growth (Hüve et al., 2007), a possible contribution to the nighttime methanol peak due to a transient increase in leaf growth following light/dark transitions (Poiré et al., 2010) might be considered. Moreover, in addition to high nighttime methanol emissions, accounting for as much as 30 % of the total diurnal emissions, non-negligible nighttime values were obtained for $G_s$ and Tr as well (Figures 3.2b and 3.2c). Nighttime emissions, co-occurring with non-negligible stomatal conductance and/or transpiration rates have been reported previously for some other plant species such as *Populus deltoides* (Harley et al., 2007), *Gossypium hirsutum* (Hüve et al., 2007) and *Fagus sylvatica* (Hüve et al., 2007; Folkers et al., 2008; Schade et al., 2011). Nighttime transpiration due to incomplete stomatal closure has also been reported for many C3 and C4 species (Caird et al., 2007). Therefore, a rather constant non-negligible stomatal conductance during the dark period, as observed in our experiments (0.02 mol $m^{-2}$ $s^{-1}$), in combination with a continuous methanol production, could be an additional pathway for the nighttime methanol emission from maize plants of stage 1.

In contrast to the maize plants of stage 1, nighttime values of methanol emissions, photosynthesis, transpiration and stomatal conductance were very low for the leaves of stage 2 and stage 3 (Figure 3.2e-i). Nighttime methanol emissions accounted for only 3 % of the total diurnal emissions, which is about 10 times less than for the fully enclosed plants of stage 1. Furthermore, the methanol emission peaks following light/dark transitions, which were so prominent for the plants of stage 1, no longer appeared in the diurnal methanol emission pattern of the leaves of stage 2 and stage 3. This is in line with the total absence of guttation on leaves of stages 2 and 3.

### 3.3.4 Leaf age affects the magnitude of methanol emission

Beside large differences in the methanol emission pattern, methanol emission magnitudes were also observed to vary strongly between maize plants of stage 1 and maize leaves of stages 2 and 3 (Figure 3.2). Whereas, in the course of a day, the emissions ranged between 0.4 and 2.3 nmol $m^{-2}$ $s^{-1}$ (between 3 and 16 µg $g_{DW}^{-1}$ $h^{-1}$) for the plants of stage 1, variations between 0 and 0.1 nmol $m^{-2}$ $s^{-1}$ (0 to 0.4 µg $g_{DW}^{-1}$ $h^{-1}$) were measured for the leaves of stages 2 and 3.

Although the magnitude of methanol emissions from leaves of stages 2 and 3 was found to be very similar (Figures 3.2f and 3.2i), it should be noted that 3 of the 5 individual leaves of stage 3 that were enclosed for analysis showed some discoloration at their edges (yellow/brown). The discoloration was an indication of the onset of the senescence process, which is rather common for leaves of this age. The health condition of those leaves was also reflected in reduced photosynthesis and leaf transpiration. The methanol emission patterns of the less healthy leaves of stage 3, however, did not vary significantly from those of the healthy leaves of stage 3, neither in shape nor in magnitude. This suggests that possible reductions in the methanol emissions by the enclosed leaf due to a reduction of the healthy part of the leaf might be compensated for by an enhanced production of methanol in the senescent part of the leaf.

When expressed per leaf area, total/daytime (i.e. PPFD > 0)/maximum methanol emissions are lower for the leaves of stages 2 and 3 than for the plants of stage 1 by a factor of 17/12/13 (Figure 3.2c, 3.2f and 3.2i). The difference between total and daytime emissions is explained by the large nighttime contribution to the emissions from the plants of stage 1. As the specific leaf area (SLA = leaf area per unit of dry mass) of leaves of stages 2 and 3 (0.034 $m^2$ $g_{DW}^{-1}$)

differs from the one of leaves of stage 1 (0.062 $m^2$ $g_{DW}^{-1}$), these methanol emission ratios will increase by a factor of 1.8 when expressing the methanol emission rates per leaf dry weight.

A reduction in methanol emission rates along with the maturity of the leaf is a well-known phenomenon which is related to the decrease in leaf growth with time and which has been reported by several other researchers (MacDonald and Fall, 1993; Nemecek-Marshall et al., 1995; Galbally and Kirstine, 2002; Hüve et al., 2007; Aalto et al., 2014). Reduction levels found in our study are in line with previous measurements on other crop, broadleaf and needleleaf species, which have been recently compiled by Wells et al. (2012).

However, even though the observed reduction in methanol emissions with leaf age is in line with the existing literature, the methanol emission rate data for the leaves of stages 2 and 3 that were observed in the present study differ quite strongly from the ones in other maize studies (Table 3.2).

Table 3.2: Methanol fluxes from this work and previous studies on maize, and corresponding measurement scale, plant age, and environmental conditions.

| | This study | | | McDonald and Fall (1993) | Graus et al. (2013) | Das et al. (2003) | Bachy et al. (2016)[d] |
|---|---|---|---|---|---|---|---|
| | stage 1 | stage 2 | stage 3 | | | | |
| measurement scale | plant/leaf | leaf | leaf | leaf | leaf | ecosystem | ecosystem |
| plant age(days) | 4-14 | 30-40 | 60-70 | * | 68 | 39 | 73-96 |
| analytical instrument | PTR-MS | PTR-MS | PTR-MS | GC-MS | PTR-MS | GC-FID | PTR-MS |
| measurement technique | enclosure measurements in a temperature and light controlled environmental chamber | | | in situ enclosure measurements | in situ enclosure measurements | flux-gradient technique | DEC-MS[e] |
| PPFD (µmol $m^{-2}$ $s^{-1}$) | 0-600 | 0-600 | 0-600 | 350 | 1000 | 300-950 | 500-700 |
| T (°C) | 23-27 | 23-27 | 23-27 | 30 | 30 | 24-28 | 23-27 |
| average $E_{CH3OH}$ [µg $g_{DW}^{-1}$ $h^{-1}$] | 5.7±0.7[a] (7.1±0.8)[b] | 0.17±0.03[a] (0.35±0.07)[b] | 0.20±0.04[a] (0.37±0.06)[b] | 45.7±2.7 | 3.74 | 34.50±14.56[c] | 0.75±0.12 |

[a] daily average emission rate.

[b] average emission rate at maximum PPFD (600 $\mu$mol $m^{-2}$ $s^{-1}$).

[c] taking into account a biomass dry weight of 100 g per $m^2$ soil surface area (Das et al., 2003).

[d] This study was carried out over an entire growth season, but only data from 73-96 days old maize plants (developmental stage R1 in Bachy et al. 2016) and within the indicated PPFD and temperature range were considered for averaging in order to allow a comparison with methanol emission rates from stage 2 and 3 leaves in this study. During this period the leaf area index (LAI) still

increased from 4.38 to 5.04 $m^2$ leaf / $m^2$ soil. The data were originally measured in $\mu$g $m^{-2}$(soil) $h^{-1}$ and were converted into $\mu$g $g_{DW}^{-1}$(leaf) $h^{-1}$ by dividing the emissions by the LAI (measured on site) and the specific leaf weight (estimated at 29 g per $m^2$ leaf ) (A. Bachy, personal communication).

[e] DEC-MS: Disjunct Eddy Covariance by Mass Scanning.

[*] youngest fully developed leaves, plant age not reporte.

In the laboratory study of MacDonald and Fall (1993) and the field study of Das et al. (2003) methanol emission rates as high as 46 and 35 µg $g_{DW}^{-1}$ $h^{-1}$ have been observed, respectively. In contrast, a more recent field study by Graus et al. (2013) resulted in emission rates of 3.74 µg $g_{DW}^{-1}$ $h^{-1}$. In our study the maximum average daytime emission rate for the leaves of stages 2 and 3 was only 0.4 µg $g_{DW}^{-1}$ $h^{-1}$. Such disagreement amongst the data can have several reasons, such as differences in maize varieties, plant physiological conditions and the actual conditions under which plants are grown (e.g. light, temperature, soil type, soil fertility and soil moisture content). Although still smaller by a factor 2, our results for stage 2 and 3 maize leaves at maximum PPFD agree better with the recent eddy covariance methanol flux measurements obtained over a maize field (same variety as in this study) in Belgium (Bachy et al., 2016) at similar light and temperature and plant developmental conditions. Comparison of leaf scale and ecosystem scale measurements, however, is not straightforward and the difference in methanol flux values with Bachy et al. (2016) could have several reasons such as the simultaneous contribution from growing and fully mature leaves, emissions from the soil and other parts of the plant (flowers, fruit), and the variation of light and temperature in the canopy. Because of these important differences amongst studies, more experiments are definitely required in order to constrain methanol emissions from this crop species.

## 3.4 Conclusions

This study revealed strong differences in the response of methanol emissions to varying PPFD between maize plants of stage 1 (young) and maize leaves of stage 2 (almost fully grown) and stage 3 (fully grown). Methanol emissions for the plants of stage 1 showed a continuous rise towards a maximum in the course of the day which was followed by a decline towards the end of the photoperiod. This indicates that the production was modulated by a response to some other

factor than light and temperature, such as a diurnal variation in leaf growth rate or PME activity. On the other hand, methanol emissions from leaves of stages 2 and 3 closely followed changes in PPFD, transpiration and stomatal conductance. They were also reproduced fairly well by the dynamic BVOC emission model of Niinemets and Reichstein when a production function dependent on both PPFD and temperature was used.

In contrast to leaves of stages 2 and 3, plants of stage 1 were characterized by non-negligible nighttime emissions of methanol and by the occurrence of large peaks following light/dark transitions. Simultaneous observations of guttation droplets on maize leaves of stage 1 suggested guttation as a hypothetical source of this emission peak. This hypothesis was strengthened by a quantification of the methanol content in the guttation fluid. Similar concentrations of methanol were also found in the guttation fluid of young winter wheat (*Triticum aestivum* L.) plants. Future studies on other plant species may be useful to better evaluate the significance of this additional methanol emission pathway.

The present study also confirmed that methanol emission from plants of stage 1 is larger than the one from leaves of stages 2 and 3 by an order of magnitude. This supports the current hypothesis that methanol emission from plants of stage 1 is strongly related to leaf growth. Emission rates in our study were rather low compared to other studies, but in fair agreement with a recent whole growing season ecosystem scale study, carried out above a maize field in Belgium and using the same maize variety as in the present study. A comparison of the flux measurements from leaves of stage 2 and stage 3 also indicated that the onset of senescence in highly mature leaves of stage 3 is an additional source of methanol.

In conclusion, this study shows that methanol emissions from maize plants are complex and differ strongly between developmental stages. It turns out little is yet understood of the emissions from plants of stage 1 in particular. Additional growth chamber and field studies, covering a wide range of environmental conditions and possibly also involving other maize varieties and crop species at different developmental stages, will be required in order to develop accurate methanol emission algorithms for agricultural crops.

# S3 Supplementary information

## S3.1 Metrics for describing plant development

In Table S3.1 of the manuscript, details were provided about the plants and the enclosed leaf/leaves at the different plant developmental stages at which the experiments were carried out. Here we provide some supplementary information about two useful metrics for describing plant development for which data are shown in the Table S3.1: the BBCH code and the accumulated growing degree-days (AGDD).

### *A. The BBCH code*

The BBCH scale, which is extensively described in Meier (2001), is a system for uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species. The abbreviation BBCH stands for "Biologische **B**undesanstalt, **B**undessortenamt und **CH**emische Industrie".

The developmental stages of the plants that were investigated in the present study (stages 1, 2 and 3) all encompassed several BBCH growth stages. The decimal BBCH codes associated to the plant developmental stages, along with a short description, are provided in Table S3.1.

Table S3.1: Plant developmental stages with BBCH codes.

| Plant stage | BBCH code | Description |
|---|---|---|
| 1 | 10 | First leaf through coleoptile |
| | 11 | First leaf unfolded |
| | 12 | 2 leaves unfolded |
| | 13 | 3 leaves unfolded |
| | 14 | 4 leaves unfolded |
| 2 | 17 | 7 leaves unfolded |
| | 18 | 8 leaves unfolded |
| | 19 | 9 leaves unfolded |
| 3 | 65 | Male: upper and lower parts of tassel in flower<br>Female: stigmata fully emerged |
| | 67 | Male: flowering completed<br>Female: stigmata drying |
| | 69 | End of flowering: stigmata completely dry |

*B. Accumulated growing degree-days (AGDD)*

In agricultural sciences, the development of crops is often described in terms of growing degree-days (GDD [°C day]). The canonical form for calculating GDD is given by Eq. S3.1 (Mcmaster, 1997):

$$GDD = \left(\frac{T_{max} + T_{min}}{2}\right) - T_{base} \quad \text{(S3.1)}$$

In this formula $T_{min}$ and $T_{max}$ are the daily minimum and maximum temperatures in the growth chamber, respectively and $T_{base}$ is the temperature below which the organism does not grow or grows very slowly (10°C for maize).

The accumulated growing degree-days parameter (AGDD) is the cumulative sum of growing degree-days after putting the seeds in the soil.

1. Equations used for modelling methanol fluxes from maize leaves and model options

Methanol flux data from maize leaves have been evaluated against the dynamic BVOC emission model of Niinemets and Reichstein (Niinemets and Reichstein, 2003a and 2003b), using a pre-defined methanol production function. Methanol in leaves is assumed to be stored in a gaseous and a liquid pool, with respective pool sizes $S_G$ and $S_L$. The pool size dynamics are governed by the differential equations S3.2a and S3.2b, in which I is the rate of methanol production, $F_m$ is the diffusion flux from the site of methanol synthesis to the outer surface of the cell walls and F is the diffusion flux from the gas phase pool to the ambient air (Niinemets and Reichstein, 2003a).

$$\frac{dS_L}{dt} = I - F_m \quad \text{(S3.2a)}$$

$$\frac{dS_G}{dt} = F_m - F \quad \text{(S3.2b)}$$

By combining Eq. (4), (6), (7), (9) and (10) from Niinemets and Reichstein (2003a), $F_m$ and F can be written as $\alpha S_L$-$\beta S_G$ and $\gamma S_G$, respectively, where α, β and γ are given by Eq. S3.3a, S3.3b and S3.3c, respectively.

$$\alpha = G_L\left(\frac{A}{f_w V}\right) \quad \text{(S3.3a)}$$

$$\beta = \left(\frac{G_L}{H}\right)\left(\frac{A}{f_{ias} V}\right) RT \quad \text{(S3.3b)}$$

$$\gamma = \left(\frac{G_G}{P}\right)\left(\frac{A}{f_{ias} V}\right) RT \quad \text{(S3.3c)}$$

In those equations $G_L$ [mol $m^{-2}$ $s^{-1}$] and $G_G$ [mol $m^{-2}$ $s^{-1}$] are the liquid phase and gas phase diffusion conductances for methanol, respectively. $G_G$ was calculated from Eq. (4), (5) and (6) in Niinemets and Reichstein (2003b), taking into account our measured values of the stomatal conductance $G_S$. The parameters $f_w$ and $f_{ias}$ are the aqueous and the gaseous fraction of the leaf volume, respectively, A is the leaf surface area [$m^2$] and V is the leaf volume [$m^3$], P is the air pressure [Pa], R is the ideal gas constant (8.314 J $mol^{-1}$ K), T is the measured leaf temperature [K], and H is the temperature dependent Henry's law constant [Pa $m^3$ $mol^{-1}$]. Leaf structural characteristics and corresponding gas phase conductances for $CO_2$ for *Phaseolus vulgaris* L. from Table 1 in Niinemets and Reichstein (2003b) were used for $G_G$ and $G_L$ calculations. As methanol is expected to be produced within the cell walls only the diffusion conductance in that compartment was considered for the calculation of $G_L$.

As described in Sect. 3.2.5 of the manuscript, the methanol emission rate given by Stavrakou et al. (2011) was used as the methanol production rate I in Eq. S3.2a. This production rate is given by Eq. S3.4a, which is a combination of Eq. (3.4) and (3.5) of the current manuscript.

$$I = \varepsilon \cdot [(1 - LDF) \cdot \gamma_{T-li} + LDF \cdot \gamma_P \cdot \gamma_{T-ld}] \quad \text{(S3.4a)}$$

In Eq. S3.4a, ε [mol $m^{-2}$ $s^{-1}$] is the production rate at standard conditions (303 K and a PPFD of 1000 µmol photons $m^{-2}$ $s^{-1}$), $\gamma_{T\text{-}ld}$ (Eq. S3.5a) is the temperature response for the light-dependent fraction of the production, $\gamma_{T\text{-}li}$ (Eq. S3.6) is the temperature response for the remainder of the production that is not light dependent, $\gamma_P$ (Eq. S3.7) is the light response for the light-dependent fraction of the production, and LDF is the light-dependent fraction of the production at standard conditions.

$$\gamma_{T-ld} = E_{opt} \cdot [C_{T2} \cdot e^{C_{T1} \cdot x} / (C_{T2} - C_{T1} \cdot (1 - e^{C_{T2} \cdot x}))] \quad \text{(S3.5a)}$$

$$x = \left[\left(\frac{1}{T_{opt}} - \frac{1}{T}\right)\right] / 0.00831 \quad \text{(S3.5b)}$$

$$\gamma_{T-li} = \exp(\beta(T - 303)) \quad \text{(S3.6)}$$

$$\gamma_P = C_P \cdot [(\alpha \cdot PPFD) / ((1 + \alpha^2 \cdot (PPFD^2)^{0.5})] \quad \text{(S3.7)}$$

In Eq. S3.5a and S3.5b, T is the leaf temperature [K] and $E_{opt}$, $T_{opt}$, $C_{T1}$ and $C_{T2}$ are 1.61, 313 K, 60 and 230, respectively (Stavrakou et al., 2011). The β-factor in Eq. S3.6 was set to 0.08 $K^{-1}$ (Stavrakou et al., 2011; Harley et al., 2007). Values of 1.066 and 0.0027 were used for $C_P$ and α, respectively, in Eq. S3.7 (Guenther, 1997).

After substitution of Eqs. S3.3a-c and Eq. S3.4a into Eqs. S3.2a-b, the coupled set of differential equations was solved with Matlab® using the ordinary differential equation solver ode15s, resulting in diurnal variations of the gas-phase pool size $S_G$, from which the methanol fluxes F could then be calculated.

The different model options that were investigated in the present work were as follows:

- The LDF parameter in Eq. S3.4a was set to zero to simulate a light independent methanol production and ε was optimized by fitting the model to the experimental data.
- The LDF parameter in Eq. S3.4a was set to 0.8 as was the case in the methanol emission function reported by Stavrakou et al. (2011) and ε was optimized by fitting the model to the experimental data.
- The model was run over a large range of LDF and ε and optimal values for those parameters were obtained by least-square fitting of the model results to the experimental data.
- For some model runs, the liquid water content of the leaves was artificially increased by dividing (A/V) in Eqs. S3.3a-c by a factor 4 (see Sect. 3.3.2 in the manuscript for further details).

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# Chapter 4: Oxygenated volatile organic compound emissions from senescent maize leaves and comparison with other leaf developmental stages

Abstract

Plants are the major source of Biogenic Volatile Organic Compounds (BVOCs) which have a large influence on atmospheric chemistry and the climate system. Therefore, understanding BVOC emissions from all abundant plant species at all developmental stages is very important. Nevertheless, investigations on BVOC emissions from even the most widespread agricultural crop species are rare and mainly confined to the green healthy leaves. Senescent leaves of grain crop species could however be an important source of BVOCs as almost all the leaves senesce on the field before being harvested. For these reasons, BVOC emission measurements have been performed on maize (*Zea mays* L.), one of the highly cultivated crop species in the world, at all the leaf developmental stages. The measurements were performed in controlled environmental conditions using dynamic enclosures and proton transfer reaction mass spectrometry (PTR-MS). The main compounds emitted by senescent maize leaves were methanol (49% of the total accumulated emission on a molar basis), acetic acid (24%), acetaldehyde (12%), acetone/propanal (5%) and hexenals (4%). Important differences were observed in the temporal emission profiles of the compounds, and both yellow leaves during chlorosis and dry brown leaves after chlorosis were identified as important BVOC sources. Total accumulated BVOC emissions from senescent maize leaves were found to be among the highest for senescent grass species. BVOC emission intensity and pattern varied strongly among the different leaf developmental stages, and senescent leaves showed a larger diversity of emitted compounds than leaves at the other stages. Methanol was the compound with the highest emissions for all the leaf developmental stages and the contribution from the young-growing, mature and senescence stage to the total methanol emission by a maize leaf was 60, 17 and 23%, respectively. Except for an eddy covariance field study conducted on the same maize variety, the BVOC emission rates obtained in this work were much lower than the scarcely available and strongly varying literature data. Further investigations are therefore required to better constrain BVOC standard emission factors from this and other highly cultivated crop species.

## 4.1 Introduction

Plants are the major source of Biogenic Volatile Organic Compounds (BVOCs) (Guenther et al., 2006) which have a large influence on atmospheric chemistry, climate and ecosystem functioning (Atkinson, 2000; Laothawornkitkul et al., 2009; Pacifico et al., 2009). Therefore, understanding BVOC emissions from all abundant plant species at all developmental stages, including leaf senescence, is very important. Leaf senescence is a very common phenomenon in nature especially in autumn for the polycarpic plants and in the harvesting season for the monocarpic plants. It is the final stage of leaf development (Lim et al., 2007; Gan and Amasino, 1997; Woo et al., 2013) that facilitates the remobilization of nutrients that are produced during the growth phase of the leaf to the developing seeds or other parts of the plant (Woo et al., 2013; Keskitalo, 2005; Gan and Amasino, 1997). Leaf senescence is mainly regulated by the developmental age, although it could be affected by environmental stresses like drought, oxidative stress (enhanced ozone and UV-B irradiation), pathogen infestation, nutrient deficiency, high or low temperature, and internal factors like phytohormones and reproductive development. Chloroplast degradation of the mesophyll cells is the first step in leaf senescence and can be easily identified by leaf chlorosis (Lim et al., 2007).

Although numerous studies have already been performed on biogenic volatile organic compounds (BVOC) emissions from healthy and growing leaves where cells were developing (Kuhn et al., 2002; Harley et al., 2007; Hüve et al., 2007; Folkers et al., 2008; Bracho–Nunez et al., 2011; Mozaffar et al., 2017), studies on senescent leaves where cells are breaking down (Gan and Amasino, 1997) are very rare. As far as we know, there is only one leaf-scale study, performed under controlled conditions (Holopainen et al., 2010), in which VOC emissions from undetached senescent leaves (of *Betula pendula Roth*) have been measured, but the measurement frequency was too low (1 GC-MS sample every 3 days) to well represent the emission dynamics. Therefore, additional studies at increased time resolution are required for a better characterization of BVOC exchanges between senescent leaves and the atmosphere during the whole senescence period. Although BVOC studies on detached and artificially senescing leaves with high time resolution have already been mentioned in the literature (Kirstine et al., 1998; Warneke et al., 1999; de Gouw et al., 1999, de Gouw et al., 2000, Karl et al., 2001b, Karl, 2005; Crespo et al., 2013), the obtained emissions may have been biased due to faster proteolysis

(protein breakdown), chlorophyll and sugar disappearance and respiration rates in those investigated leaves compared to attached senescent leaves (Thimann et al., 1974).

To assess the importance of BVOC fluxes from the senescent leaves of a plant, information about BVOC fluxes from other leaf developmental stages (young, semi-mature, mature) is also necessary. In this study we will focus on BVOC emissions from maize (*Zea Mays L.*) leaves at those different stages. Despite being a vastly cultivated crop species worldwide, few literature studies have been devoted to VOC emissions for that species (MacDonald and Fall, 1993; Das et al., 2003; Graus et al., 2013; Bachy et al., 2016; Mozaffar et al., 2017) and none of them cover all the leaf developmental stages. In particular, BVOC flux data from senescent maize leaves are totally missing in the abovementioned literature.

Maize is a monocarpic plant and its organismal senescence occurs with seed maturation (Lim et al., 2007). However, the first leaf at the base of the plant starts senescing much before flowering and this process continues for all the leaves from the base to the top of the plant throughout the growing season. Therefore, emissions from senescent leaves could provide a significant contribution to the total BVOC emission budget from a maize field, as suggested by de Gouw et al. (2000).

In this paper we will present BVOC emissions from senescent maize leaves and also from other leaf developmental stages, and provide answers to the following questions: 1) which BVOCs are emitted during the senescence process and in what proportions, 2) how do BVOC emissions from senescent maize leaves compare to those from other grass species, 3) how do BVOC emissions vary among the different leaf developmental stages of maize, and 4) what are the contributions of the different developmental stages to the total methanol emission by a maize leaf/plant?

## 4.2 Methods and materials

### 4.2.1 Plants and environmental conditions

Experiments were performed on maize leaves (*Zea mays* L., variety Prosil, Caussade Semences, France) at four different leaf developmental stages: young, semi-mature, mature, and senescent. To measure BVOC exchanges between young leaves and the atmosphere, 8 to 14 days old maize plants with fast growing young leaves were used. The almost fully developed and healthy 7$^{th}$

leaves of 30-35 days old plants, and the fully developed 7th leaves of 55-60 days old plants were used for sampling BVOC fluxes from semi-mature and mature leaves, respectively. More information about the young, semi-mature, and mature leaves can be found in Mozaffar et al. (2017). The senescent $7^{th}$ leaves of about 60-95 days old plants were used to investigate BVOC fluxes at this final stage. Investigations on young, semi-mature, and mature leaves were performed on 5 replicates for each stage, and 10 replicates were used for investigating senescent leaf emissions. All these experiments were conducted on potted (20L pots filled with a mixture of 75% silty clay loam soil and 25% sand) plants in a temperature and light controlled environmental chamber. Details about the environmental chamber can be found in Mozaffar et al. (2017). The soil was fertilized before plantation with NPK fertilizer (6-5-5, Substral nutrimax, Belgium) containing micronutrients (Cu, Fe, Mn, Mo, Zn) as well. Daytime temperature in the environmental chamber was maintained at 25 °C throughout the experimental period and during the night temperature reduced by around 2 °C due to absence of a heat source. Seventeen hours of photoperiod at three different PPFD (Photosynthetic Photon Flux Density) values (100, 330 and 600 µmol $m^{-2}$ $s^{-1}$) were alternated by seven hours of darkness (Fig. 4.1). All the plant replicates were watered regularly to maintain a good soil moisture content (35-40%, field capacity was 40%).

### 4.2.2 Experimental setup

Twenty-two L cylindrical dynamic flow-through enclosures made of transparent PFA (perfluoroalkoxy Teflon) envelope (Norton, Saint-Gobain Performance Plastics, NJ, USA) were used to enclose the young maize shoots. Similar 30L enclosures were used to partially enclose the semi-mature, mature, and senescent leaves. In addition, a similar empty enclosure was used as a reference to measure the background VOC concentrations. The enclosures were equipped with a Teflon fan, a thermistor (type 10k, NTC, Omega, UK), and a relative humidity sensor (type HIH-3610, Honeywell, NJ, USA ) for proper mixing of air and continuous monitoring of leaf temperature, air temperature and relative humidity, respectively. Relative humidity and $CO_2$ concentration in the 5 SLM (standard liter per minute) purge air flow through each of the enclosures were controlled at around 40% and 400 ppm, respectively. A conventional quadrupole-based Proton Transfer Reaction-Mass Spectrometer (hs-PTR-MS, Ionicon Analytik GmbH, Innsbruck, Austria) and a LI-7000 non-dispersive IR gas analyser (LI-COR, Lincoln,

Nebraska, USA) were used to determine BVOC, $H_2O$ and $CO_2$ concentrations. In general, BVOC and $H_2O$ calibrations were performed every 3-5 days. Details about the set-up have been presented in Mozaffar et al. (2017).

### 4.2.3 Tentative identification and quantification of emitted BVOCs

The quadrupole-based hs-PTR-MS instrument was operated at a drift tube pressure and temperature of 2.1 hPa and 333 K, respectively, and at an E/N value (ratio of the electric field to the air number density in the drift tube) of 130 Td (1Td = $10^{-17}$ V $cm^2$). Measurements were carried out in the Multiple Ion Detection mode, in which the instrument continuously cycled through a list of preset m/z values. The list included m/z 21 and m/z 39, which correspond to isotopes of the reactant ion species $H_3O^+$ and $H_3O^+H_2O$, respectively, and BVOC-related m/z values for which ion signals above the detection limit have been observed in PTR-MS spectra of maize leaf emissions. The detection limit is defined here as twice the standard deviation of the background ion signal, obtained from zero air measurements. The subset of m/z values which were used for flux quantification, along with a tentative identification of the associated BVOCs is shown in Table 4.1.

Table 4.1: The m/z ratios and tentative identification of those compounds for which fluxes were quantified. Compounds that were present in the multi-component calibration bottles are indicated in bold and their mixing ratios (±5% accuracy) in the bottles are shown as well. Compounds that are underlined have been unambiguously identified by Karl et al. (2005) as VOCs released from drying rice (*O. sativa*) by using the hyphenated GC/PTR-MS technique.

| m/z | Compound | Dwell time (s) | Mixing ratio (ppbv) | |
|---|---|---|---|---|
| | | | Bottle 1[a] | Bottle 2[a] |
| 33 | **methanol** | 10 | 1074 | 1013 |
| 45 | **acetaldehyde** | 2 | 999 | 969 |
| 59 | **acetone,** propanal, 2,3-butanedione | 2 | 973 | 502 |
| 61 | acetic acid | 2 | | |
| 69 | **isoprene**, pentenol, n-pentanal | 2 | 441 | 483 |

| | | | | |
|---|---|---|---|---|
| 73 | **methyl ethyl ketone** (MEK), 2-methyl-propanal, butanal | 2 | 494 | 516 |
| 81 | **monoterpenes, hexenals** | 2 | 851[c] | 1041[b] |
| 83 | n-hexanal, **hexenols**, hexenyl acetates | 2 | | 986[d] |
| 137 | **monoterpenes** | 2 | 851[c] | |

[a] Calibration bottle 1 was used for the experiments with young, semi-mature and mature leaves. Bottle 2 was used for the senescence experiments.

[b] trans-2-hexenal was used as a proxy for hexenals and was the only compound resulting in m/z 81 product ions in calibration bottle 2

[c] alpha-pinene (452 ppbv) and sabinene (399 ppbv) were used as a proxy for the monoterpene family and were the only compounds resulting in m/z 81 product ions in calibration bottle 1

[d] only the cis-3-hexenol isomer was present in the calibration bottle

PTR-MS calibrations were performed by using gravimetrically prepared multi-component mixtures of BVOCs in nitrogen (Apel-Riemer Environmental Inc., Denver, CO, USA) with a certified accuracy of 5%. The calibration gas was further diluted with zero air, generated by sending ambient air through a catalytic converter (Parker® ChromGas® Zero Air Generator, model 1001, Parker Hannifin Corporation, Haverhill, MA, USA), to obtain mixing ratios in the lower ppbv range. One step calibrations were performed for the BVOCs, since both the one step and multiple steps calibration procedures resulted in the same calibration factor. When several compounds contributed to the ion signal at a given m/z value, it was assumed that they possessed similar calibration factors. By calibrating the PTR-MS to the compound indicated in bold in Table 4.1 and applying the calibration factor to the measured ion signal at that m/z, we then estimated the total concentration of those compounds. Two calibration bottles were used to allow quantification of monoterpenes in the experiments with young, mature, and semi-mature leaves, and hexenals in the experiments with senescent leaves. The sum of the ion signals at m/z 81 and

137, weighed for their relative transmission in the mass spectrometer, was used to determine fluxes of the sum of all emitted monoterpene isomers from the young, semi-mature and mature leaves. Ions signals at m/z 137 were below the detection limit for senescent leaves, indicating that monoterpene fluxes were insignificant during this stage. Ions at m/z 81 therefore did not originate from monoterpenes and were only attributed to hexenals. In order to obtain accurate concentrations for the sum of hexenal isomers, the other main proton transfer product ions of hexenals at m/z 57 and m/z 99 (Pang, 2015) were continuously monitored as well and the sum of the three product ion species, weighed for ion transmission, was used to derive fluxes of the sum of hexenal isomers. The calibration factor for acetic acid (at m/z 61), a compound which was not present in the calibration bottles, was estimated from the one for acetone (at m/z 59) by considering the fragmentation of the protonated molecules in the drift tube (Inomata and Tanimoto, 2010; Schwarz et al., 2009) and the ratio of the calculated collision rate constants for the proton transfer reactions (Su, 1994) and by assuming the same PTR-MS transmission efficiency for ions at m/z 59 and 61. In what follows, the m/z 81 and 83 compounds emitted by the senescent leaves will be designated as GLVs.

### 4.2.4 Flux computation

Unless explicitly mentioned otherwise, BVOC fluxes from maize leaves are expressed as molar fluxes per unit of leaf surface area [mol $m^{-2}$ $s^{-1}$]. Molar BVOC fluxes from leaves ($F_{BVOC}$) are obtained by multiplying the molar flow rate of the purge air through the plant enclosure ($F_{air}$) by the difference between the BVOC mole fractions in the sampled air from the plant enclosure output ($x_{BVOC,PE}$) and the empty reference enclosure output ($x_{BVOC,RE}$). The mole fractions, in turn, are obtained by dividing the normalized PTR-MS BVOC ion signals $I_{m/z}$ [ncps] by the BVOC calibration coefficients $C_{BVOC}$ [ncps $ppbv^{-1}$] (Mozaffar et al., 2017).

The area of the enclosed leaves was measured regularly for the young, semi-mature, and mature leaves (Mozaffar et al., 2017). During senescence, the maize leaves shrank and rolled inward due to drying and leaf area was changing continuously. Consequently, the initially enclosed leaf area was used in the flux estimations from senescent leaves.

#### 4.2.4.1 Correction of acetaldehyde fluxes for interferences of $CO_2$ exchange

Despite having a smaller proton affinity than $H_2O$, production of protonated $CO_2$ still occurred to some extent in the PTR-MS and contributed to the ion signal at m/z 45 which is used for acetaldehyde flux calculation. In order to quantify the contribution of $HCO_2^+$ to the m/z 45 ion signal, air was sampled sequentially from two empty enclosures and controlled flows of pure $CO_2$ were added to the BVOC-free purge air in one of the enclosures. A calibration coefficient $C_{CO2}$ could then be determined from the linear relationship between the differences in m/z 45 ion signal ($\Delta I_{45}$) and in $CO_2$ mixing ratio ($\Delta X_{CO2}$) in the sampled air from the two enclosures ($C_{CO2}=\Delta I_{45}/\Delta X_{CO2}$).

Since a fraction of the $CO_2$ in the purge flow is taken up by the enclosed plant/leaf as a result of photosynthesis, the $CO_2$ concentration in the sampled air from the plant enclosure will change in the course of the day and the instantaneous acetaldehyde flux will be given by:

$$F_{acetaldehyde} = F_{air} \times \left( \left( I_{45,PE} - I_{45,RE} \right) - \left( X_{CO2,PE} - X_{CO2,RE} \right) \times C_{CO2} \right) / C_{acetaldehyde} \quad \text{(Eq.4.1)}$$

Due to the contribution of protonated $CO_2$ at m/z 45, apparently negative acetaldehyde fluxes had to be corrected for $CO_2$ uptake by the leaf during photosynthesis. As this $C_{CO2}$ calibration coefficient has not been determined during the experiments with young, semi-mature and mature maize leaves, acetaldehyde fluxes were only obtained for senescent leaves. Note however that this correction was only important at the onset of the senescence process when leaf photosynthesis was still significant.

### 4.3 Results and discussion

#### 4.3.1 BVOC emissions from senescent maize leaves

Healthy maize leaves were enclosed a few days before the onset of chlorosis and BVOC emissions were monitored as the senescence progressed. The process of chlorosis, during which the leaf first turned yellow and then brown, started at the tip of the leaf and gradually progressed towards the base of the enclosed leaf within 5-10 days (10 replicates). During and after the chlorosis period the brownish parts of the leaf turned inward and curled while completely drying out. Significant emissions of methanol, acetaldehyde, m/z 59 compounds, acetic acid and

hexenals were observed. Small fluxes of m/z 69, m/z 73 and m/z 83 compounds were noticed too, but they hardly exceeded the flux detection limit ($5.24\times10^{-12}$, $2.5\times10^{-12}$, and $5.01\times10^{-12}$ mol $m^{-2}$ $s^{-1}$, respectively). BVOC emissions emerged or increased (in case the BVOC was also emitted constitutively) at the onset of chlorosis. While some of the BVOCs were predominantly emitted during and just after the chlorosis period, others were emitted from dead biomass as well (will be discussed in detail in the following sections). Photosynthesis and transpiration started to decrease a few days before the start of chlorosis, diminished gradually as chlorosis progressed towards the base of the leaf and finally stopped 2-4 days before the end of chlorosis (Fig. 4.1). The temporal emission dynamics of the individual BVOC species, the relative contribution of their cumulative emissions, as well as a comparison with cumulative emissions from other senescent grass species will be discussed hereafter.

#### 4.3.1.1 Methanol, acetaldehyde and GLVs are predominantly emitted during the chlorosis period

At the beginning of the senescence process, the emission rates of methanol, acetaldehyde and hexenals rose slowly as leaf discoloration moved onward from the tip to the base and they increased faster when the leaf was turning brown and was shrinking due to the drying process. The highest emissions occurred at around 1-3, 0-2 and 0-2 days before the end of the chlorosis period, respectively (Fig. 4.1). By then photosynthesis and transpiration from the steadily decreasing green part of the enclosed leaf were already firmly reduced. Emissions of methanol, acetaldehyde and GLVs exceeding 20% of their maximum value lasted for 3-8, 3-7 and 2-7 days, respectively. The variability in the duration of the high emission periods for the different compounds reflects the variability in temporal emission profiles for the 10 replicates. Fig. 4.1 also shows small methanol and acetaldehyde emissions after the chlorosis period which persisted until the end of the measurements. Those emissions showed a fixed diurnal pattern with a maximum emission rate of at most 10% of the maximum emission rate during the chlorosis period. The temporal evolution of normalized m/z 83 compound fluxes was similar to that of normalized hexenal fluxes (data not shown).

As can be noticed in Fig. 4.1, no distinct diurnal patterns were observed for methanol, acetaldehyde and GLV fluxes during the chlorosis period and there was no clear correlation between those fluxes and the environmental conditions (temperature, PPFD) in the dynamic

enclosures. In addition, all these compounds showed high nighttime fluxes. Fall et al. (1999) also observed light independent GLV emissions from detached dried out beech (*Fagus sylvatica* L.) and clover (*Trifolium repens* L.) leaves, and Warneke et al. (1999) mentioned temperature independent emissions of methanol from drying beech leaves. Furthermore, nighttime emissions of methanol and acetaldehyde for drying alfalfa (*Medicago sativa* L.) were also noticed by Warneke et al. (2002).

The link between the evolution of the senescent leaf and the temporal emission profile suggests that the biochemical and physical mechanisms involved in leaf chlorosis and drying are major drivers of BVOC emission during leaf senescence. Presumably collapse of the cellular structure during drying (Karl et al., 2001a; Karl et al., 2001c; de Gouw et al., 1999) and disintegration of cell organelles and dying cells (Keskitalo et al., 2005) are at the origin of high emissions of these compounds. As the maximum emissions were noticed when there was hardly any photosynthesis and transpiration, the stomata of a senescent leaf at this phase may have remained closed even in the presence of light (Ozuna et al., 1985). Therefore, the main emission route for methanol and acetaldehyde during this senescence phase was probably no longer diffusion through the stomata, as is the case for healthy leaves (MacDonald and Fall, 1993; Kreuzwieser et al., 2000; Rottenberger et al., 2004). Degradation of the epidermal layer could be a potential emission route during this late senescence phase as changes in the biomechanical properties of the epidermis cuticle have been documented (Takahashi et al., 2012).

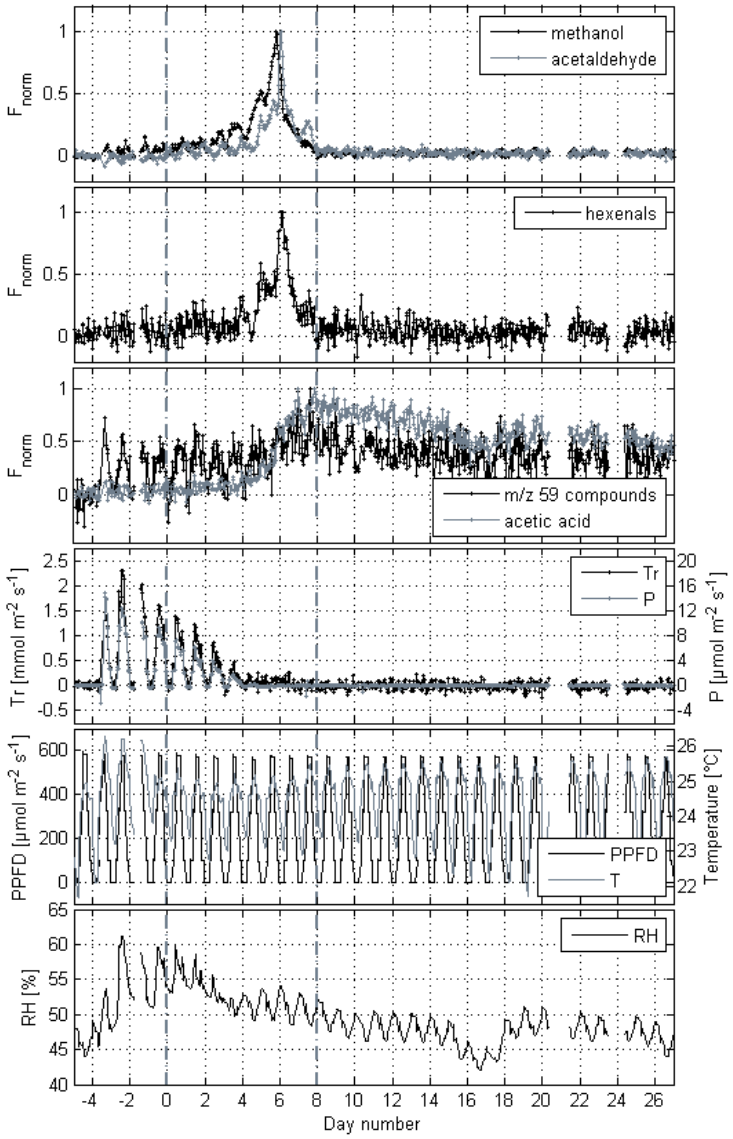


Figure 4.1: Normalized fluxes of methanol, acetaldehyde, hexenals, m/z 59 compounds and acetic acid from a senescent maize leaf together with the transpiration rate (Tr), the photosynthesis rate (P), light (PPFD), temperature (T) and relative humidity (RH) conditions in the enclosure. The BVOC fluxes have been normalized with respect to their maximum value to facilitate the comparison of temporal emission profiles for the different compounds. The dot-dashed vertical grey lines indicate the start and the end of the chlorosis period. Gaps in the data are due to instrument failure. As the temporal evolution of senescence and consequently also of BVOC fluxes varied considerably

among the leaf replicates, data from a representative replicate are shown in Fig. 4.1 to illustrate emission dynamics instead of averaged data over all replicates.

In leaves, methanol is mainly produced by pectin demethylation in the cell wall, catalysed by pectin methylesterase (PME) enzymes (Fall and Benson, 1996; Fall 2003), and this process occurs during growth as well as during aging and senescence of plant tissues (Nemecek-Marshall et al., 1995). Due to continuous breakdown of cellular materials for nutrient remobilization during senescence (Woo et al., 2013; Keskitalo, 2005), massive changes occur in the primary cell wall structure, which potentially stimulates massive methanol production. A possible reason for the methanol emissions from the dead, brown leaves after the chlorosis period could be the presence of active PME in dead plant materials (Castaldo et al., 1997; Galbally and Kirstine, 2002). Most of the PME formed by the plant remains active under normal environmental conditions and is capable of demethylation of about 65% of the pectin in the dead plant material (Galbally and Kirstine, 2002).

The acetaldehyde production mechanism in senescent leaves is not yet well-established, but fatty acid peroxidation by reactive oxygen species (ROS) has been suggested by Jardine et al. (2009) as a potential mechanism based on their $^{13}C$ isotope analysis studies on stressed leaves of deciduous trees. As increased oxidative stress and lipid peroxidation have been observed (Prochazkova et al., 2001) during maize leaf senescence, the suggested mechanism might be the reason of the high acetaldehyde emissions observed in the present study beside collapse of the cellular structure as mentioned before.

GLV emissions from senescent leaves have been reported previously in the literature. While Holopainen et al. (2010) mentioned increases in GLV emissions from senescent leaves of *Betula pendula Roth* before abscission, others observed hexenal and hexenol emissions from leaf drying experiments (Fall et al., 1999; Fall et al., 2001; de Gouw et al., 2000; Karl et al., 2001a; Karl et al., 2001c; Karl et al., 2001b; Warneke et al., 2002; Karl et al., 2005). During leaf senescence, most of the cellular fatty acids are oxidized to supply energy for the senescence process (Lim et al., 2007). Since fatty acid oxidation, catalysed by 13-lipoxygenase (13-LOX), is the initial step in GLV production in leaves (Scala et al., 2013; Hatanaka et al., 1993), high GLV emissions can be expected during leaf senescence if 13-LOX is available and active. Furthermore, recent research revealed the key role of this enzyme in the degradation of chloroplasts during leaf

senescence (Springer et al., 2016), potentially leading to strong increases in the emission of GLVs with increasing chlorosis of the leaf.

#### 4.3.1.2 Persistent m/z 59 compounds and acetic acid emissions from dead leaves

Whereas the temporal evolution of acetic acid emissions was well-marked, m/z 59 compounds emissions from senescent leaves showed a much smaller increase with respect to the constitutive emissions by the end of the chlorosis period (Fig. 4.1). Normalized acetic acid and m/z 59 compounds emissions increased somewhat slower than those of acetaldehyde and GLVs, resulting in a delay of their maximum emissions with respect to the latter two compounds by 1-2 days.

Furthermore, in strong contrast to methanol, acetaldehyde and GLVs, acetic acid and m/z 59 compounds fluxes remained close to their maximal value for a few days after the end of the chlorosis period and then slowly decreased with time, but never reached zero during the entire measurement period. Early morning acetic acid emissions were generally somewhat lower than at the end of the day, presumably due to a decrease in temperature in the leaf enclosures during the night. A similar diurnal behaviour was observed for the m/z 59 compounds emissions from senescent leaves which, in contrast to the pre-senescence constitutive emissions, remained well above the zero level in dark conditions. High day- and nighttime m/z 59 compounds emissions (acetone) from dry plant parts and litter have previously been mentioned in the literature (Schade and Goldstein, 2001; Warneke et al., 2002) and acetic acid emissions from dry plant material have been reported as well (Kesselmeier et al., 1998; Warneke et al., 1999; Crespo et al., 2013). The production of acetone and acetic acid during the drying process following the chlorosis period might be explained by the occurrence of a non-enzymatic Maillard reaction (Ikan et al., 1996). After the end of the chlorosis period a significant positive correlation ($R^2$=0.53, P<0.01) was observed between the acetic acid emissions and the relative humidity of the purge air. Similarly, Warneke et al. (1999) observed a large increase in partially oxidized VOC (POVOC) emissions after wetting of dried biomatter. They explained this by the transfer of some of the POVOC molecules, produced by the Maillard reaction and remaining attached to the surface of the solid structure of the dry leaf material, to the aqueous phase through replacement by highly polar water molecules. The dissolved POVOCs were subsequently transferred from the aqueous to the gas phase until a gas/liquid equilibrium, determined by the Henry's law constant, was

reached. Although the dry leaves were not wetted in our experiments, release of acetic acid molecules from the biomass surface layer may have been influenced by the presence of a relative humidity dependent microlayer of water on the surface layer or by interactions with polar water vapor molecules which may have promoted the release of the polar BVOC molecules. A short additional experiment in which an inert surface, coated with a pure acetic acid microlayer, was inserted in an empty enclosure demonstrated that such a positive correlation between acetic acid emissions and relative humidity was not restricted to senescent biomass. Indeed, the acetic acid emission rate from the coated surface, which was of similar magnitude as the one from enclosed senescent maize leaves, was found to increase by a factor 2.7 when increasing the relative humidity of the purge air from 40 to 64 %. Moreover, increased emissions of acetic acid and other polar compounds with increasing relative humidity have been reported previously from other materials such as wood boards (Steckel et al., 2013) and indoor furniture (Fechter et al., 2006; Schaeffer et al., 1996) as well.

#### 4.3.1.3 Absolute BVOC emissions from senescent maize leaves and relative contribution of the emitted compounds to the total BVOC emission from senescent leaves

Total accumulated emissions of methanol, acetaldehyde, m/z 59 compounds, acetic acid, and hexenals from senescent maize leaves were 130±30, 33±9, 14±8, 64±23, and 10±8 µmol $m^{-2}$, respectively, and the corresponding maximum emission rates were $62\pm32\times10^{-5}$, $18\pm5\times10^{-5}$, $1.6\pm0.5\times10^{-5}$, $5.8\pm1.4\times10^{-5}$, and $4\pm2\times10^{-5}$ µmol $m^{-2}$ $s^{-1}$ (Table 4.2). Since the emission rates for some compounds (e.g. methanol, m/z 59 compounds, and acetic acid) were still well above the detection limit ($1.36\times10^{-11}$, $1.91\times10^{-12}$ and $3.54\times10^{-12}$ mol $m^{-2}$ $s^{-1}$, respectively) at the end of the measurement period, the accumulated emission values from the onset of chlorosis to the end of the measurement period (27 days) for those compounds should be considered as lower limits for the total emissions from the senescent leaves.

Table 4.2: BVOC fluxes from maize leaves of different developmental stages. Maximum values for the instantaneous ($E_{inst,max}$) and daily flux ($E_{day,max}$), accumulated flux values from the onset of chlorosis to the end of the measurement period ($E_{acc,tot}$, accumulation over 27 days) and the contribution of the accumulated flux over the chlorosis period to the total accumulated flux ($E_{acc,chlorosis}$/ $E_{acc,tot}$) are shown for the different BVOCs emitted during senescence. Also tabulated are daily fluxes ($E_{day}$) from young, semi-mature and mature maize leaves. Data for the senescent leaves are averages over 10 replicates and data for the young, semi-mature and mature leaves are averages over 5 replicates.

| | senescent leaves | | | | young leaves | semi-mature leaves | mature leaves |
|---|---|---|---|---|---|---|---|
| Compounds | $E_{inst,max}$ ($10^{-5}$ µmol $m^{-2}$ $s^{-1}$) | $E_{acc,total}$ (µmol $m^{-2}$) | $E_{acc,chlorosis}$/ $E_{acc,total}$ (%) | $E_{day,max}$ (µmol $m^{-2}$ $day^{-1}$) | $E_{day}$ (µmol $m^{-2}$ $day^{-1}$) | $E_{day}$ (µmol $m^{-2}$ $day^{-1}$) | $E_{day}$ (µmol $m^{-2}$ $day^{-1}$) |
| Methanol | 62±32 | 130±30 | 75 | 36±19 | 69±9 | 3.8±0.8 | 4.3±0.9 |
| Acetaldehyde | 18±5 | 33±9 | 62 | 7±2 | - | - | - |
| m/z 59 | 1.6±0.5 | 14±8 | 30 | 0.8±0.3 | 0.8±0.3 | 0.22±0.10 | 0.5±0.2 |
| Acetic acid | 5.8±1.4 | 64±23 | 19 | 4.2±1.0 | <DL* | <DL | <DL |
| m/z 69 | 2.0±0.4 | 8±4 | 39 | 0.8±0.2 | <DL | <DL | <DL |
| m/z 73 | 1.0±0.6 | 4±5 | 35 | 0.3±0.3 | 0.4±0.2 | 0.15±0.10 | 0.12±0.03 |
| Hexenals | 4±2 | 10±8 | 61 | 2.0±1.4 | - | - | - |
| Monoterpenes | <DL | <DL | - | <DL | 1.2±0.7 | 0.13±0.05 | 0.08±0.05 |
| m/z 83 | 1.6±0.5 | 4±2 | 53 | 0.5±0.3 | - | - | - |

* Detection Limit

Methanol was clearly the compound with the highest emission, accounting for almost half (49%, emissions are expressed in molar units) of the total accumulated emission from the onset of leaf chlorosis to the end of the investigations (Fig. 4.2a). It was followed by acetic acid (24%), acetaldehyde (12%), m/z 59 compounds (5%) and hexenals (4%). The m/z 69, m/z 73 and m/z 83 compounds also contributed a small fraction to the total BVOC fluxes from the senescent leaves, but their individual contributions were not more than 3%.

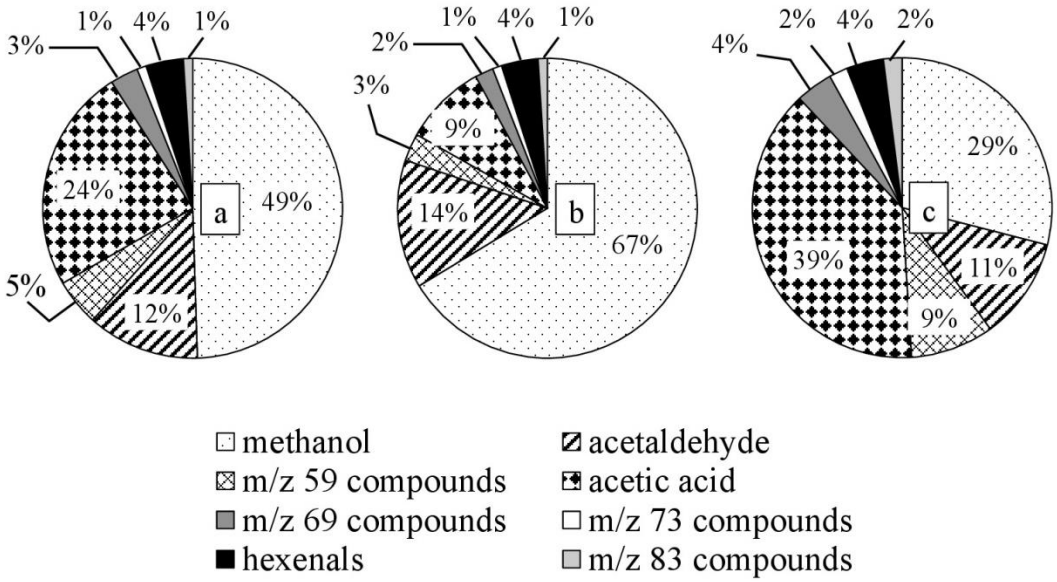


Figure 4.2: Proportion of accumulated BVOC fluxes from the senescent leaves for different periods: (a) from the onset of leaf chlorosis to the end of the investigations, (b) during leaf chlorosis, (c) during a period of 5 days starting about 15 days after the end of chlorosis (totally brown leaves). Accumulated emissions were averaged over 10 replicates and only compounds which individually made up over 0.5% of the total BVOC emission were considered.

Fig. 4.2 clearly shows that the relative contribution of the emitted compounds varied among the time periods considered. Differences in relative BVOC composition reflect the differences in temporal emission dynamics among BVOCs that were discussed in Section 4.3.1.1 and 4.3.1.2. Indeed, as accumulated emissions from methanol, acetaldehyde and hexenals were dominant during the chlorosis period (b) (see Table 4.2), it is clear that those compounds together make up a large part (85%) of the BVOC emissions for that subperiod. Because of the strong persistence of m/z 59 compounds and acetic acid emissions after the chlorosis period, the relative

contribution of those compounds increases with time after that period. At the end of the measurements period (Fig. 4.2c), the m/z 59 compounds and acetic acid together even make up almost half (48%) of the BVOC emissions, and acetic acid was predominantly emitted at that time (39%).

While some studies (Karl et al., 2001b; Karl et al., 2005; Eller et al., 2011; Crespo et al., 2013) have reported accumulated BVOC emissions from biomass drying and simulated drying/senescence experiments, information about accumulated BVOC emissions from senescent leaves that are still attached to the plant is, to our knowledge, not available in the literature. Consequently, as the abovementioned studies are the only available ones performed in conditions which somehow approximate senescence in real natural conditions, we will compare our results from senescent maize leaves against the results from those studies (Table 4.3), which were all performed on different grass species. Overall, BVOC emissions from senescent maize leaves are among the highest for the drying/senescent grass species. The integrated methanol emission from the senescent maize leaves is very similar to the one from hay, reported by Karl et al. (2001b). In contrast, integrated methanol emissions from *Sorghum sudanense* (sorghum) (Karl et al., 2005), *Oryza sativa* (rice) (Karl et al., 2005), *Panicum virgatum* (switchgrass) (Eller et al., 2011), and *Phyllostachys nigra* (black bamboo) (Crespo et al., 2013) are several orders of magnitude lower than those for maize and hay. Accumulated emissions of acetaldehyde, m/z 59 and m/z 69 compounds from maize are of the same order of magnitude as those from drying hay and black bamboo, and considerably higher than those from sorghum, rice, and switchgrass. Total acetic acid emission from maize is several fold higher than that from black bamboo, which is the only other grass species for which accumulated acetic acid emissions have been reported (Warneke et al. (1999) also mentioned acetic acid emission from grass, but didn't report integrated emissions). Maize, hay, rice, and black bamboo all have higher integrated GLV emissions than sorghum and switchgrass. Differences can be explained by species dependency, by the possible differences in production and emission mechanisms from dead (excised drying plants) and living plant materials, and differences in meteorological/environmental conditions.

Table 4.3: Comparison of the integrated BVOC emissions during senescence of maize leaves and drying of hay, *Sorghum sudanense* (sorghum), *Oryza sative* (rice), *Panicum virgatum* (switchgrass) and *Phyllostachys nigra* (black bamboo). Also mentioned in the Table are the integration period and the temperature at which the experiments were performed.

| | | Maize | Hay | Sorghum | Rice | Switchgrass | Black bamboo |
|---|---|---|---|---|---|---|---|
| | | Current study | Karl et al. (2001b) | Karl et al. (2005) | | Eller et al. (2011) | Crespo et al. (2013) |
| | | ± total (~27 days) | total* | total (7-9 hours) | total (7-9 hours) | partial** (1 day) | total (3 days) |
| | | 25 °C | 25-35 °C | 30 °C | 30 °C | 30 °C | 80 °C |
| Compound | m/z | (µg/$g_{DW}$) | (µg/$g_{DW}$) | (µg/$g_{DW}$) | (µg/$g_{DW}$) | (µg/$g_{DW}$) | (µg/$g_{DW}$) |
| Methanol | 33 | 143±34 | 160 | 2±0.9 | 3.1±0.8 | 9.07±2.35 | 26.1 |
| Acetaldehyde | 45 | 49±14 | 20-80 | 6.6±2 | 8.4±0.7 | 13.99±5.44 | 73.2 |
| Acetone/propanal | 59 | 28±15 | 20-40 | 0.4±0.3 | 1.7±1.1 | 2.21±0.69 | 13.3 |
| Acetic acid | 61 | 132±47 | - | - | - | - | 28.6 |
| C5-compounds | 69 | 24±13 | 15[a] | 0.32±0.13[b] | 3.2±1.7[b] | 1.15±0.53[c] | 58[c] |
| MEK/butanal | 73 | 10±13 | 11-80 | - | - | - | - |
| Hexenals | 57/81/99 | 36±27 | 100-240 | 0.53±0.17 | 100±49 | 1.49±1.42 | 231[d] |
| Hexenols | 83 | 13±9 | 30-60[e] | 0.36±/20.17 | 34±13 | 1.06±1.49 | |

[a] pentenol and 2-methyl-butanal; [b] pentenol and n-pentanal; [c] pentenol and isoprene; [d] based on the sum of ions intensities at m/z 57, 81, 99, 83, 101 and 103; [e] hexenols and hexanal. *instantaneous emission values reached zero level at the end of the integration period; **instantaneous emission value did not reach zero level at the end of the integration period.

### 4.3.2 Comparison of BVOC emissions from young, semi-mature, mature and senescent maize leaves

BVOC fluxes have also been determined for young and fast growing, semi-mature, and mature maize leaves in order to assess their relative importance with respect to those of senescent maize leaves. A comparison of the diurnal flux patterns and intensities of methanol, m/z 59 compounds, m/z 73 compounds and monoterpenes for the different developmental stages of maize leaves will be discussed in Section 4.3.2.1 and 4.3.2.2 and fluxes obtained in this work will be compared with available literature data in Section 4.3.2.3. As explained in Section 4.2.4.1, acetaldehyde fluxes could not be calculated from the m/z 45 ion signals for the young, semi-mature and mature stages due to interferences of protonated $CO_2$ which were not quantified during those experiments. Diurnal flux patterns of acetic acid and m/z 69 compounds for these 3 stages are not shown either because the fluxes almost never exceeded the detection limit.

#### 4.3.2.1 Methanol fluxes

Methanol fluxes from young, semi-mature and mature maize leaves have been extensively described in a previous paper (Mozaffar et al., 2017) and will only be briefly discussed here. For reasons of completeness, however, the corresponding diurnal flux patterns are shown in Fig. 4.3(a, g and m) as well. Methanol was the BVOC with the highest emission rate for all developmental stages. Daily fluxes from young, fast-growing leaves, expressed in molar units (nmol $m^{-2}$ $s^{-1}$), were found to be around 17 times higher than those of semi-mature and non-growing mature leaves (Table 4.2). This is in line with previous observations on other plant species (MacDonald and Fall, 1993; Nemecek-Marshall et al., 1995; Galbally and Kirstine, 2002; Hüve et al., 2007; Aalto et al., 2014) and is related to the enhanced production of methanol in leaves by the PME-catalysed demethylation of pectin within plant cell walls during leaf growth (Fall and Benson, 1996; Fall 2003). As this process also takes place during cell wall remodeling in senescent leaves, methanol production increased again in this period and maximum daily

methanol fluxes during the chlorosis period were around half the daily fluxes from young leaves (Table 4.2). The diurnal methanol emission pattern also varied strongly between the leaf developmental stages as elaborately described in Mozaffar et al. (2017) for the first three developmental stages and in Section 4.3.1 for the senescent stage.

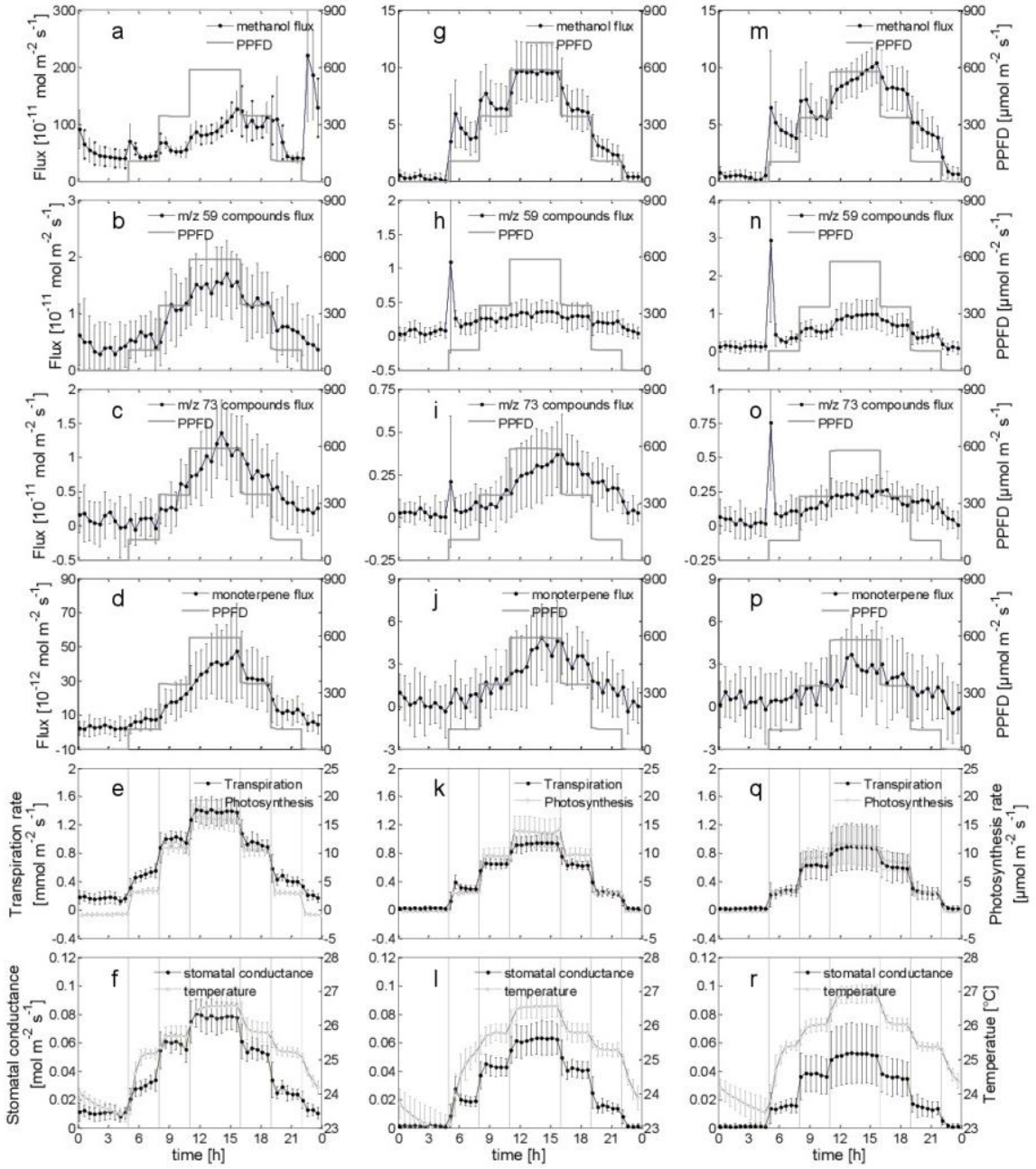


Figure 4.3: Diurnal flux pattern of methanol, m/z 59, m/z 73 compounds and monoterpenes, along with photosynthesis, transpiration, stomatal conductance and the

light and temperature conditions in the dynamic enclosures for experiments with young (a-f), semi-mature (g-l) and mature (m-r) maize leaves. The large error bars (standard deviation) on the fluxes are mainly due to plant-to-plant variability. Vertical dashed grey lines in the last two rows of figures indicate the time of the stepwise PPFD increases/decreases. Note the different scales in the primary y-axes of the first four figure rows.

#### 4.3.2.2 Fluxes of m/z 59 compounds, m/z 73 compounds, and monoterpenes

Similar as for methanol, the maximum daily fluxes for m/z 59 and m/z 73 compounds from the senescent leaves and the daily fluxes from young leaves were higher than the daily fluxes from semi-mature and mature leaves, but only by a factor 1.6-3.6 (Table 4.2). Higher acetone emission rates were also observed from growing needles of *Pinus sylvestris* L. than from mature needles (Aalto et al., 2014). Bracho-Nunez et al. (2011) reported higher acetone emission rates from young leaves of some Mediterranean plant species (*Cistus albidus*, *Pinus halepensis* and *Prunus persica*) compared to mature leaves and suggested that this might be related to the higher requirements of young leaves for defense. However, this leaf age effect is not a general trend as the opposite was found for some other Mediterranean plant species, which indicates that the dependence of acetone emissions on leaf development is highly variable among species.

Except during senescence, the variations in m/z 59 and m/z 73 compound fluxes from maize leaves followed variations in PPFD (Fig. 4.3 (b, h, n and c, i, o)), albeit often with some delay. Furthermore, semi-mature and mature leaves showed a large transient flux increase when turning the lights on in the growth chamber. A similar phenomenon has been observed for methanol for young, semi-mature and mature leaves at all stepwise increases in PPFD, as can be noticed in Fig. 4.3 (a, g and m), and has been described in detail in Mozaffar et al. (2017). Those transient emissions can be explained by the fast OVOC transfer from the liquid to the gas phase OVOC pool in the leaf and its subsequent diffusion through the stomata as a result of a temporary disequilibrium between those pools, induced by a sudden increase in stomatal conductance. The rate of equilibration strongly depends on the aqueous OVOC solubility and increases with increasing Henry's law constant (Niinemets and Reichstein, 2003a; Niinemets and Reichstein, 2003b). The Henry's law constants of acetone (3.88 Pa $m^3$ $mol^{-1}$ at 25 °C, Sander, 2015) and MEK (5.77 Pa $m^3$ $mol^{-1}$ at 25 °C, Sander, 2015), which are expected to be the main contributors

to m/z 59 and m/z 73 compounds, respectively, are higher than the one of methanol (0.461 Pa $m^3$ $mol^{-1}$ at 25 °C, Sander, 2015). Consequently the duration of the transient emission for m/z 59 and m/z 73 compounds should be shorter than for methanol, which is in agreement with the observations (see Fig. 4.3). Transient increases of acetone emission in the morning have been reported for some other plant species as well (Brilli et al., 2014; Bourtsoukidis et al., 2014).

Significant monoterpene emissions were only observed from young, semi-mature and mature leaves, whereas for the senescent leaves it was below the detection limit. Daily emissions from the young leaves were about a magnitude higher than those from the semi-mature and mature ones (Table 4.2). The reduction of monoterpenes emission with the age of the leaf has been already mentioned by several other investigators (Bracho-Nunez et al., 2011; Aalto et al., 2014; Hakola et al., 1998; Kuhn et al., 2004). Lower defense demands by the mature leaves (Bracho-Nunez et al., 2011) and low metabolic activity in mature leaves resulting in low de novo synthesis (Aalto et al., 2014) have been put forward as potential reasons for this decrease or loss of monoterpenes emission potential. Variations in monoterpene fluxes were positively correlated with variations in PPFD for all three stages but, similar as for m/z 59 and m/z 73 compounds, the flux increases were delayed with respect to the PPFD increases.

#### 4.3.2.3 Comparison with previous BVOC studies on maize

Apart from the present study, only three BVOC flux studies investigated other compounds than methanol for maize (*Zea Mays* L.). Our study is the only one focusing on all leaf developmental stages. Fluxes (in µg $g_{DW}^{-1}$ $h^{-1}$) of methanol, m/z 59 and m/z 73 compounds, and monoterpenes from the different studies at similar light and temperature conditions are shown in Table 4.4. As discussed in Mozaffar et al. (2017), the methanol emission rate at maximum PPFD for mature leaves in the present study was lower than the one reported for mature leaves by Graus et al. (2013) and Das et al. (2003) by 1 and 2 orders of magnitude, respectively. A better agreement (factor 2 difference) was found with eddy-covariance flux measurements performed in Belgium on the same maize variety as the one used in the present study (Bachy et al., 2016).

Similar as for methanol, the m/z 59 compounds and monoterpenes emission rate at maximum PPFD for mature maize leaves in the current study was much lower (8-19 times) than those reported by Graus et al. (2003) and by Das et al. (2013) (Table 4). However, the flux ratio of m/z

59 compounds (acetone) to methanol (in mass units) in those studies (0.12 and 0.15, respectively) is very similar to the one observed in our environmental chamber study (0.16). Conversely, the m/z 59 compounds flux from mature leaves in our study were in good agreement with those obtained in the ecosystem scale study of Bachy et al. (2016). A similar monoterpenes/methanol mass flux ratio was observed for mature leaves in this work (0.08) and in the study of Graus et al. (2013) (0.10) for leaves at similar conditions. This ratio was twice as large in the study by Das et al. (2003). The average monoterpene emission rate from mature leaves at maximum PPFD in the present work was of the same absolute magnitude as the one in the field study of Bachy et al. (2016) at similar conditions, but of opposite sign, indicating a small net deposition in this ecosystem scale study. Similar as for methanol and acetone, the m/z 73 compounds emission rate for mature leaves at maximum PPFD in the present study was found to be significantly lower (factor 6.5) than the one reported in the study of Graus et al. (2013).

The overall conclusion of this comparison with literature data is that large discrepancies in emission rates exist among the different studies for the different BVOCs emitted by mature leaves. Further studies are therefore required to better constrain BVOC emissions from this important crop species. Moreover, caution is required when comparing leaf scale and ecosystem scale measurements as the latter may be affected by contributions from leaves of different developmental stages and facing different environmental conditions throughout the canopy, contributions from other vegetative parts of the plants like stems and flowers and contribution from soil.

Table 4.4: Average methanol, m/z 59, m/z 73 and monoterpenes fluxes from maize leaves obtained in this study at maximal PPFD (600 $\mu$mol $m^{-2}$ $s^{-1}$) and comparison with fluxes obtained in other maize studies at similar light and temperature conditions.

| | This study | | | Graus et al. (2013) | Das et al. (2003) | Bachy et al. (2016)[b] |
|---|---|---|---|---|---|---|
| | Young | Semi-mature | Mature | | | |
| measurement scale | plant/leaf | Leaf | Leaf | Leaf | Ecosystem | Ecosystem |
| plant age (days) | 4-14 | 30-40 | 60-70 | 69 | 40 | 73-96 |
| analytical instrument | PTR-MS | | | PTR-MS | GC-FID | PTR-MS |
| measurement technique | enclosure measurements in a temperature and light controlled environmental chamber | | | in situ enclosure measurements | flux-gradient technique | DEC-MS[c] |
| PPFD (µmol $m^{-2}$ $s^{-1}$) | 600 | 600 | 600 | 1000 | 300-950 | 500-700 |
| T (°C) | 27 | 27 | 27 | 30 | 24-28 | 23-27 |
| $E_{methanol}$ (µg $g_{DW}^{-1}$ $h^{-1}$) | 7.1±0.8 | 0.35±0.07 | 0.37±0.06 | 3.74±1.13 | 34.50±14.56[a] | 0.75±0.12 |
| $E_{acetone}$ (µg $g_{DW}^{-1}$ $h^{-1}$) | 0.19±0.05 | 0.03±0.01 | 0.06±0.02 | 0.57±0.19 | 4.25±2.23[a] | 0.05±0.09 |
| $E_{MEK/butanone}$ (µg $g_{DW}^{-1}$ $h^{-1}$) | 0.16±0.07 | 0.03±0.02 | 0.020±0.003 | 0.13±0.12 | - | - |
| $E_{monoterpenes}$ (µg $g_{DW}^{-1}$ $h^{-1}$) | 0.9±0.5 | 0.05±0.03 | 0.03±0.02 | 0.39±0.35 | 6.61±1.47[a] | -0.04±0.04 |

[a] taking into account a biomass dry weight of 100 g per $m^2$ soil surface area (Das et al., 2003).

[b] This study was carried out over an entire growth season, but only data from 73-96 days old maize plants (developmental stage R1 in Bachy et al. 2016) and within the indicated PPFD and temperature range were considered for averaging in order to allow a comparison with emission rates from mature leaves in this study. During this period the leaf area index (LAI) still increased from 4.38 to 5.04 $m^2$ leaf / $m^2$ soil. The data were originally measured in $\mu$g $m^{-2}$(soil) $h^{-1}$ and were converted into $\mu$g $g_{DW}^{-1}$(leaf) $h^{-1}$ by dividing the emissions by the LAI (measured on site) and the specific leaf weight (estimated at 29 g per $m^2$ leaf ) (A. Bachy, personal communication).

[c] DEC-MS: Disjunct Eddy Covariance by Mass Scanning.

#### 4.3.2.4 Contribution of senescence and other leaf developmental stages to the total methanol emission from a typical 7th maize leaf and extrapolation to a whole maize plant

By taking into account the experimental daily methanol flux data from the young and mature leaves and the accumulated methanol emissions from senescent leaves (Table 4.2), and by using information about the relative leaf area growth rate and the duration of the young-growing and mature leaf developmental stages, it was possible to estimate the accumulated methanol emission from a young-growing, mature and senescent $7^{th}$ leaf of a maize plant. Details about the estimation procedure are given in an accompanying supplementary information file. The total estimated amounts of methanol emitted by a young-growing, mature and senescent $7^{th}$ maize leaf were 11.4±0.9, 3.1±0.9 and 4.4±1.2 µmol, respectively, and the resulting total amount of methanol emitted by a $7^{th}$ leaf in the course of its lifetime is therefore 18.9 ± 1.7 µmol. The young-growing, mature and senescent stages consequently contributed 60±3, 17±5 and 23±6 % to the total methanol emission from a $7^{th}$ leaf.

Based on the emission rates obtained for a $7^{th}$ leaf, the total amount of methanol emitted by all leaves of a representative maize plant, grown in the environmental chamber at 25 °C and exposed to the diurnal light pattern described in Section 4.2.1, has been estimated for the different leaf developmental stages. Details of this estimation procedure are also provided in the supplementary information file. Values of 150±12, 30±21 and 56±17 µmol were obtained for the accumulated methanol emission from all young-growing, mature and senescent leaves over the

entire lifetime of the plant, respectively. The total amount of methanol emitted by the leaves of a whole maize plant from shoot emergence to full senescence was therefore equal to 236±30 µmol and the contributions of the different stages to the total methanol emission were 64±3, 13±9 and 24±6 %, respectively. The estimation of the total accumulated methanol emission from a whole maize plant over the course of its lifetime should be considered with caution. As already mentioned it was assumed that all leaves were exposed to the same PPFD and an effect of shading by leaves with a higher leaf number was not taken into account. Moreover, a large variability was noticed among leaves with different leaf numbers in terms of mature stage duration, which may be related to the demands of the plant for nutrient relocation. The total leaf emissions of methanol from a maize plant presented in this work could be used as a conservative estimate for upscaling to field conditions in regions characterized by environmental conditions close to those of our growth chamber. However, a general upscaling from the leaf/plant level to ecosystem level would definitely benefit from methanol emission measurements at different temperatures at all leaf developmental stages and it should be realized that deposition of methanol to the ecosystem may also be important in the field (Laffineur et al., 2012; Wohlfahrt et al., 2015).

For the other BVOCs emitted by maize plants, assumptions about the temporal evolution of fluxes at the different developmental stages are less straightforward as for methanol. Consequently, estimation of the relative contribution of those stages for a $7^{th}$ maize leaf and extrapolation to an entire maize plant from the flux data obtained in this work would have been prone to very large errors and has therefore not been accomplished.

## 4.4 Conclusions

Maize is one of the highly cultivated crop species worldwide, but only 5 studies on BVOC exchanges from maize are available in the literature and 3 of them deal with emissions from mature leaves only. Given the importance of this agricultural C4 species, we therefore undertook a growth chamber study to investigate BVOC emissions from young, semi-mature, mature, and senescent maize leaves, albeit with a strong focus on the senescent stage. In contrast to most studies on BVOC emissions from artificially senescing leaves (cutting and drying), the senescent maize leaves in our experiments were still attached to the stems, as is the case in nature.

Senescent maize leaves were found to be an important source of a wide range of BVOCs. The main emitted compounds, ranked according to their accumulated emissions over the senescence period, were found to be methanol, acetic acid, acetaldehyde, m/z 59 compounds, hexenals, m/z 69 compounds, m/z 83 compounds, and m/z 73 compounds. Important differences were observed in the temporal emission profiles of these compounds. Whereas methanol, acetaldehyde and GLVs (hexenals and m/z 83 compounds) were emitted mainly during the chlorosis period, m/z 59 compounds and acetic acid fluxes increased rather at the end of that period and their emissions remained high for a long time, even when the leaves were already completely dry. Beside m/z 59 compounds and acetic acid, the rest of the abovementioned compounds were also emitted in small but significant amounts from dry leaves after the end of the chlorosis period. Therefore, not only the yellow senescent maize leaves but also the dry brown leaves, which remain attached to the plant, are an important source of BVOCs. By comparing accumulated BVOC emissions from senescent maize leaves with other artificially senescing grass species, it was found that maize leaves were clearly among the strongest emitting grass species during that leaf developmental stage. Nevertheless, an improved comparison of accumulated BVOC emissions among senescent grass species might benefit from additional measurements on naturally senescing grass species instead of results obtained from cutting and drying experiments.

Whereas senescent leaves showed a large diversity of emitted compounds, BVOC fluxes for young, semi-mature and mature leaves which exceeded the detection limit and which could be quantified, were limited to methanol, m/z 59 and m/z 73 compounds and monoterpenes. Methanol was clearly the highest emitted compound for all stages and showed a strong variation in intensity and diurnal emission pattern among the different leaf developmental stages. The contributions from the young-growing, mature and senescent stages to the total methanol emission from a typical $7^{th}$ leaf of a maize plant were estimated to be 60, 17 and 23%, respectively.

In the present study we observed rather low BVOC emissions from mature maize leaves, in strong contrast to previous studies by Das et al. (2003) and Graus et al. (2013), but in reasonable agreement with a recent ecosystem-scale field study by Bachy et al. (2016). The very large emission rate differences for methanol, m/z 59 and m/z 73 compounds and monoterpenes among

the different studies clearly indicate that BVOC emissions from this important agricultural C4 species are still not well understood at all and deserve further experimental efforts under different meteorological conditions. Furthermore, similar investigations are required for other highly cultivated crop species to better constrain BVOC standard emission factors for use in regional and global atmospheric chemistry and climate models.

## S4 Supplementary information

Except for the experiments with young-growing maize plants, all methanol flux measurements that were reported in the present study have been carried out on 7$^{th}$ leaves of maize plants. The question therefore arises as to whether it would be possible to determine the contribution of the different developmental stages to the total methanol emission from a 7$^{th}$ leaf during its lifetime. Whereas methanol emissions from the 7$^{th}$ leaves were continuously followed during senescence, fluxes at the mature stage were only performed during limited time periods (3 consecutive days). Moreover, because of the high risk of inducing methanol emissions during leaf enclosure, flux measurements could not be performed on young 7$^{th}$ maize leaves and information on BVOC emissions from growing leaves was obtained by totally enclosing young plant shoots and measuring fluxes over six consecutive days (from day 8 to day 14 following seed germination). Further information on the experiments on young-growing plants and mature leaves can be found in a previous manuscript (Mozaffar et al., 2017). By combining all methanol flux measurements with information on the leaf area evolution of a young 7$^{th}$ leaf and the length of the growing and mature stages, the accumulated methanol emission from a 7$^{th}$ maize leaf during its entire growing, mature and senescent stage could nevertheless be estimated and the estimation procedure will be explained in the following sections. Moreover, based on the emission rates obtained for a 7$^{th}$ leaf, the total amount of methanol emitted by all leaves of a representative maize plant in the course of its lifetime has been estimated for the different leaf developmental stages.

### S4.1 Estimation of the accumulated methanol emission from the 7$^{th}$ leaf during the growth stage

Fig. S4.1 shows the variation of daily methanol fluxes $E_{day}$ [µmol m$^{-2}$ day$^{-1}$] from young fully enclosed maize shoots with the daily relative leaf area growth rate (dA/dt)/A [day$^{-1}$], A being the leaf area [m$^2$]. The average value for $E_{day}$ obtained for mature leaves (4.3 µmol m$^{-2}$ day$^{-1}$), for which the leaf area no longer changes with time ((dA/dt)/A=0), is also shown. By fitting a line through those data points, while forcing it through the data point for mature leaves, the following analytical expression (Eq. S4.1) was obtained for $E_{day}$ as a function of (dA/dt)/A for young leaves:

$$E_{day} = 285.2 \times \left(\frac{1}{A}\frac{dA}{dt}\right) + 4.3 \qquad \text{(Eq. S4.1)}$$

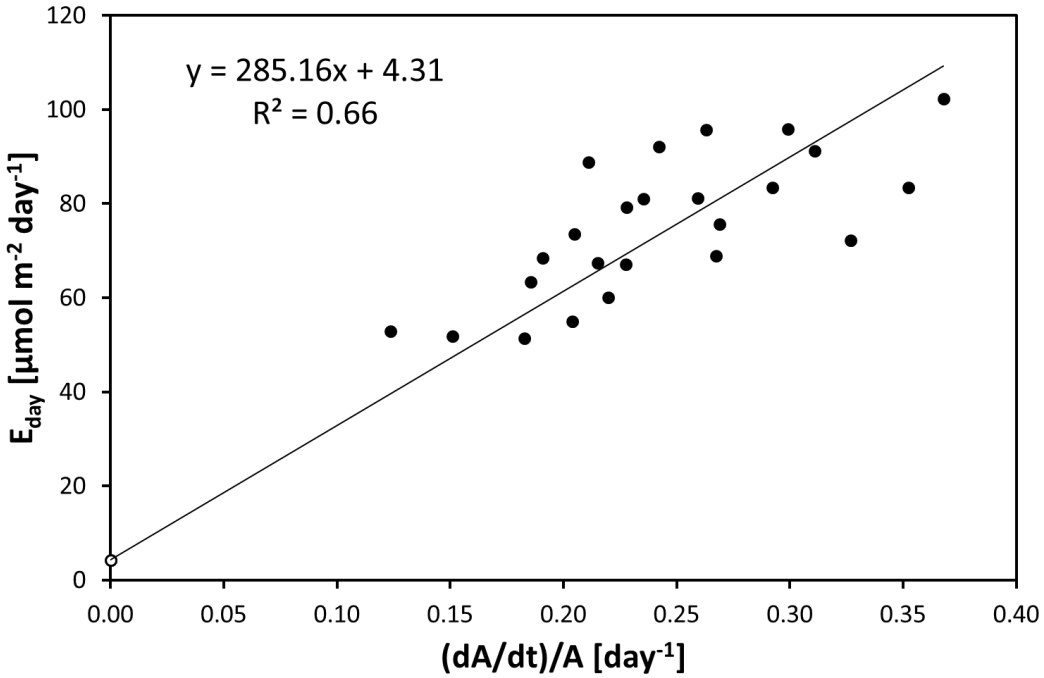


Fig. S4.1: daily methanol fluxes versus relative leaf area growth rate. The filled circles refer to different young maize plant replicates (8-14 days after germination) and the open circle is the average value over all mature maize leaves (5 replicates).

The daily methanol flux $E_{day}$ of a growing 7$^{th}$ maize leaf can be calculated from Eq. S4.1 provided the temporal evolution of the leaf area A is known. The latter can be estimated by the Gompertz function (Eq. S4.2), taking into account the appropriate values for the parameters b (5.5) and c (0.51) for a 7$^{th}$ leaf which was grown in similar conditions as in our environmental chamber (Cao et al., 1988). $A_{final}$ is the leaf area of the mature, non-growing leaf.

$$A = A_{final} exp[-bexp(-ct)] \qquad \text{(Eq. S4.2)}$$

The validity of Eq. (S4.2) for our experiments was checked by comparison against experimental leaf area data from a 7$^{th}$ leaf (Fig. S4.2a). As Eq. (S4.2) with the pre-defined b and c parameters (Cao et al., 1988) performed well in predicting the leaf area measurements, it has been applied in this paper to estimate the leaf area evolution of the young-growing 7$^{th}$ leaf and, by extension, of all young-growing leaves of an entire maize plant (see Section S4.4).

The total daily methanol emission was subsequently calculated as the product of the daily averaged leaf area (Fig. S4.2a) and the daily methanol emission (Fig. S4.2b). The cumulative methanol emission for a 7$^{th}$ leaf from emergence to maturity is shown in Fig. S4.2c.

As shown in Fig. S4.2a, it takes about 13-14 days for a normal 7$^{th}$ leaf to evolve from an emergent to a mature leaf. A slight underestimation of the leaf area by the Gompertz function during the first 2 days after leaf emergence and a 13% overestimation between days 5-10 were noticed (Fig. S4.2a), but this didn't affect the total accumulated methanol emission over the entire growing period by more than 4% (Fig. S4.2c).

As a linear relationship for the total daily methanol fluxes versus (dA/dt)/A was only established for the (dA/dt)/A range between 0 and 0.40, large uncertainties may be associated to the calculated daily methanol fluxes at (dA/dt)/A values above 0.40 which were observed for emerging leaves (first 3 days). However, since the leaf area was still small at that time, the contribution of the methanol emissions from this early period to the total accumulated methanol emission during the entire growth phase remains limited. By considering the leaf area evolution given by Eq. (S4.2) and taking into account an experimentally determined value for $A_{final}$ of 345±58 cm$^2$ (average over 10 replicates), the total amount of methanol emitted by a growing 7$^{th}$ maize leave was estimated to be 11.2±0.9 µmol.

### S4.2 Estimation of the accumulated methanol emission from the 7$^{th}$ leaf during the mature stage

As shown in Table 4.2 in the manuscript, the average daily methanol flux for a mature 7$^{th}$ leaf is 4.3±0.9 µmol m$^{-2}$ day$^{-1}$. This value was obtained for leaves at the end of the mature stage, close to senescence and does not differ by more than 10% with the daily methanol flux for semi-mature leaves, which was obtained close to the start of the mature stage. Consequently, a constant daily emission flux, equal to the one of mature leaves, was assumed for the entire duration of the mature stage. The experimentally determined duration of this stage, from zero relative leaf area increase to the onset of chlorosis, was 21±4 days (average over 10 replicates) and the experimentally determined leaf area was 345±58 cm$^2$. The estimated total amount of methanol released from a mature leaf was therefore 3.1±0.9 µmol.

### S4.3 Estimation of the accumulated methanol emission from the 7$^{th}$ leaf during senescence

The total amount of methanol emitted by a senescent 7$^{th}$ maize leaf was obtained by multiplying the accumulated flux for this period (128±28 µmol m$^{-2}$) with the experimentally determined leaf area of 345±58 cm$^2$ at the onset of chlorosis and equals 4.4±1.2 µmol.

S4.4Extrapolation of the data obtained for the 7$^{th}$ maize leaf to a whole maize plant

The accumulated methanol emission from the young-growing stage of all leaves (16 for the variety used in our experiments) of a representative maize plant, grown in the environmental chamber at the temperature and light conditions described in the manuscript (Section 4.2.1), have been estimated in the same way as was done for the 7$^{th}$ leaf (see Section S4.1). For this estimation the appropriate leaf number dependent parameters b and c (Cao et al., 1988) and the measured leaf area of the individual mature leaves ($A_{final}$) were taken into account to calculate the temporal evolution of the leaf area A of those leaves in the young-growing stage (Eq. S4.2) and consequently to estimate the corresponding daily methanol fluxes $E_{day}$ (Eq. S4.1). The estimated total amount of methanol emitted by the young-growing leaves of an entire maize plant was 150±12 µmol.

The accumulated methanol emission from all mature leaves of a maize plant in the course of its lifetime was estimated by assuming that the emission rate for all mature leaves is equal to the one of the mature 7$^{th}$ leaf (4.3±0.9 µmol m$^{-2}$ day$^{-1}$). It is obtained by multiplying the emission rate by the total area of the mature leaves (0.44±0.09 m$^2$) and by the time between the end of the leaf growth phase and the onset of leaf chlorosis. This period varies considerably between leaves (from 21±4 days for the 7$^{th}$ leaf to 10±2 days for the 10$^{th}$ and 11$^{th}$ leaf). An average value of 16±10 days was considered for the estimation, resulting in a total accumulated emission from all mature leaves of 30 ±21 µmol.

In order to estimate the total amount of methanol emitted by the senescent leaves of a whole maize plant, it was assumed that all the leaves senesce completely and that the amount of methanol emitted during senescence per unit leaf surface area is constant and equal to the one obtained for the 7$^{th}$ leaf in this study (128±28 µmol m$^{-2}$). By multiplying this value by the total mature leaf area of 0.44±0.09 m$^2$, the total amount of methanol emitted from senescent leaves of an entire maize plant is 56±17 µmol.

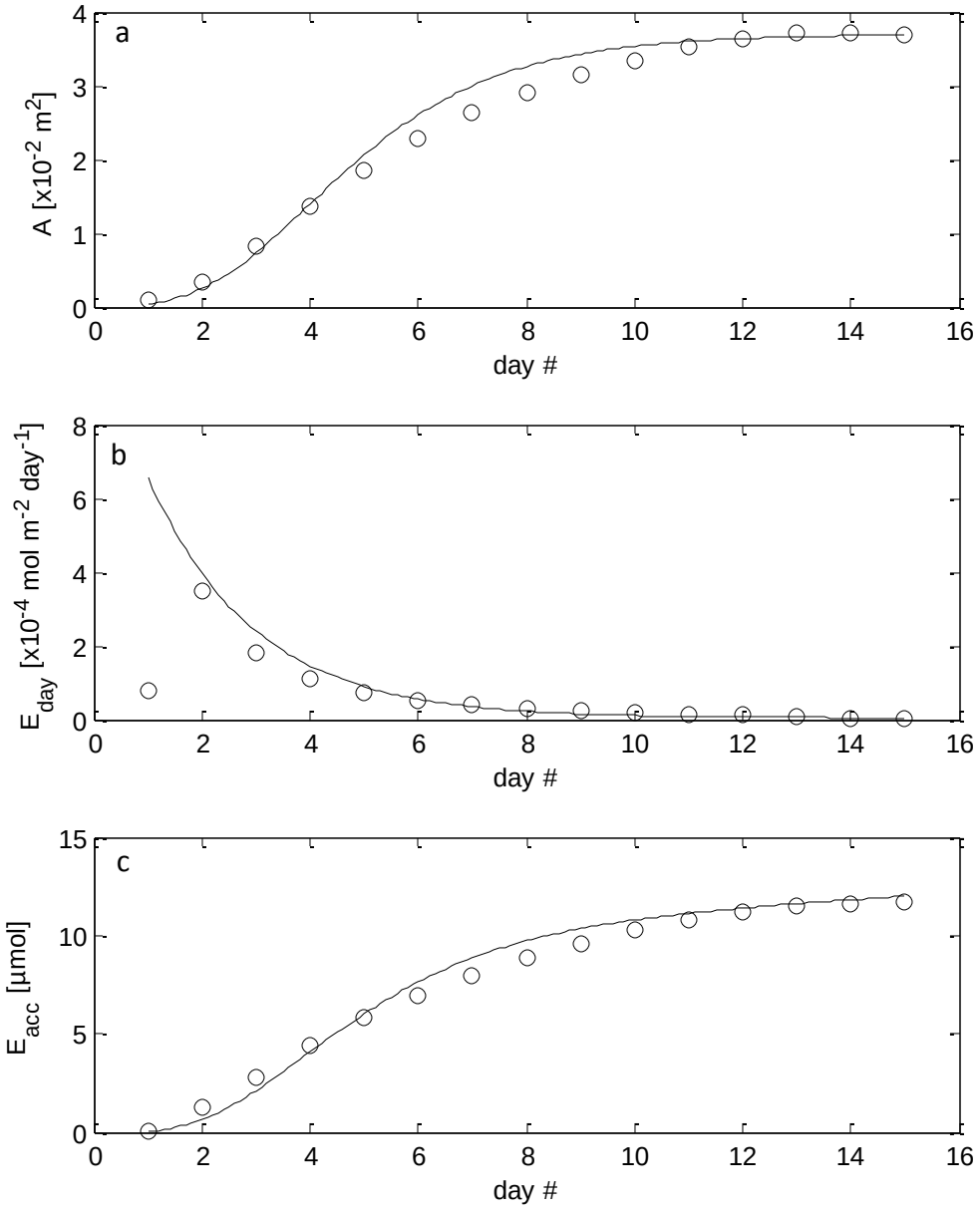


Fig. S4.2: Temporal evolution of the estimated leaf surface area (a), daily methanol flux (b) and cumulative methanol emission (c) for a typical 7$^{th}$ leaf of a maize plant. Open circles are based on actual leaf area measurements, solid lines are based on leaf area determination with the Gompertz function (Eq. S4.2).

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# Chapter 5: Grazing induced BVOC fluxes from a managed grassland

Abstract

Grazing is a common grassland management practice. Since more than half of the world's agricultural land is grazing land, this kind of biotic stress could be a potentially important source of BVOCs in the atmosphere. However, no investigations have been performed yet on grazing-induced BVOC fluxes from grassland. This is the first investigation to find out how grazing-induced BVOC fluxes differ from the constitutive ones. BVOC fluxes were followed simultaneously from side by side situated undisturbed and grazed grassland patches in a managed grassland at the Dorinne Terrestrial Observatory (DTO) in Namur, Belgium using automated dynamic flow-through enclosures and Proton Transfer Reaction-Mass Spectrometry. Strong qualitative (flux directions) and quantitative (flux strength) differences were observed between the BVOC fluxes from the grazed and undisturbed grassland patches. Among the quantified BVOCs from the undisturbed grassland, the daytime emitted compounds were methanol, acetone (only during midday), butanone/butanal, hexenals/monoterpenes (only during midday), whereas acetaldehyde, acetic acid, methyl vinyl ketone/methacrolein, hexenols/hexenyl acetates were deposited all the time. Whereas a high positive correlation ($|\rho|$=0.43 to 0.76) between fluxes of all the daytime emitted compounds and light intensity, temperature, evapotranspiration and net photosynthesis rates was observed, the deposited compound fluxes were negatively correlated ($|\rho|$=0.39 to 0.93) with ambient concentration. In contrast to the undisturbed grassland, daytime emissions were observed from the grazed grassland for all the abovementioned compounds and these induced emissions lasted for 2-5 days for the different compounds after 1 hour of grazing by cows. As a consequence, the accumulated fluxes of BVOC compounds over 7.5 days for the grazed grassland differed from those estimated for the undisturbed grassland. Nevertheless, grazing-induced fluxes observed in the current study are typically one to two orders of magnitude lower than the seasonal grassland harvest-induced fluxes (hay production) mentioned in the literature. Since grazing is a common grassland management practice on these vast ecosystems and induces high BVOC fluxes, upon proper understanding of BVOC source strength, its influences have to be incorporated in the regional and global chemistry and climate models to assess the effect of BVOC fluxes on present and future air quality and climate.

## 5.1 Introduction

Investigations of Volatile Organic Compounds (VOCs) fluxes have been an interesting topic to the atmospheric science community for a long time because of the influence of VOCs on atmospheric chemistry (Atkinson, 2000) and the climate system (Laothawornkitkul et al., 2009 and references therein; Pacifico et al., 2009 and references therein). Biogenic VOC (BVOC) emissions comprise up to 90% of the global VOC emissions (Guenther et al., 2012). Comprehensive investigations on the ecosystem/atmosphere exchange of those compounds is required because of their potential to modify the climate by forming tropospheric ozone (Atkinson, 2000), influencing the atmospheric lifetime of methane (Pacifico et al., 2009 and references therein), and affecting the formation and growth of Secondary Organic Aerosol (SAO) (Kulmala et al., 2004 and references therein; Hallquist al., 2009 and references therein).

BVOC emissions from vegetation can be either constitutive or induced by abiotic (e.g. drought, heat, flooding, mechanical wounding, air pollution) and biotic stress (e.g. herbivory damage, pest and pathogen attack), or be a combination of both. The BVOC emission intensity and composition from a stressed plant is different from a non-stressed conspecific plant (Penuelas et al., 2005; Holopainen and Gershenzon, 2010; Schaub et al., 2010). Therefore, atmosphere/plant VOC exchanges depend on the conditions of the plant, and it is necessary to conduct investigations on a plant species under all prevalent stress conditions as well as under control conditions. Several studies have already been performed on herbivore (Llusia and Penuelas, 2001; Penuelas et al., 2005) and abiotic (Bracho-Nunez et al., 2012; Bourtsoukidis et al., 2013; Jardine et al., 2015; Davison et al., 2008; Brilli et al., 2012) stress-induced BVOC emissions from different plant species. However, studies related to stress-induced BVOC emissions from agricultural ecosystems are rare.

Grassland covers one fourth of the earth's land surface (Graedel and Crutzen, 1993), 40% of the ice-free terrestrial surface (Ramankutty and Foley, 1999) and 20% of the European continent (Janssens et al., 2003). Grassland ecosystems are sources and sinks of reactive trace gases which play an important role in atmospheric chemistry and air pollution (Brunner et al., 2007; Davison et al., 2008; Brilli et al., 2012; Bamberger et al., 2010; Hörtnagl et al., 2011; Jaars et al., 2016; Ruuskanen et al., 2011). However, despite the importance of this ecosystem, the number of BVOC exchange studies that have been conducted on intact grassland (Custer and Schade, 2007;

Jaars et al., 2016) and on cutting and drying of grasses (Brunner et al., 2007; Davison et al., 2008; Brilli et al., 2012; Bamberger et al., 2010; Hörtnagl et al., 2011; Ruuskanen et al., 2011; Warneke et al., 2002; de Gouw et al., 1999; Karl et al., 2001a, b) is still limited. These last studies have already mentioned induced BVOC emissions. Beside cutting and drying of grasses, grazing by livestock is also a common grassland management practice. About 60 percent of the world's agricultural land is grazing land (FAO, 2017a) and grazing systems in the temperate zones cover about 13 percent of the world's pasture lands (FAO, 2017b). Grazing on this vast area could induce BVOC emissions by tearing and trampling. But, so far, to our best knowledge, no study has been performed on BVOC fluxes from grazed grassland yet.

In the present study, to find out the effect of grazing on grassland/atmosphere BVOC exchange, we followed BVOC fluxes from grazed and intact grassland simultaneously. We used both PTR-MS (Proton Transfer Reaction-Mass Spectrometry) and GC-MS (Gas Chromatography-Mass Spectrometry) techniques for high temporal resolution BVOC exchange measurements and for the identification of the emitted compounds, respectively. The main objectives of the current paper were to find an answer to the following questions: (i) What are the main exchanged BVOCs from undisturbed grassland and their exchange intensity and main controlling parameters, (ii) How do grazing-induced BVOC fluxes differ from the constitutive fluxes, and (iii) How do BVOC fluxes vary between two grassland management practices, i.e. rotational grazing in the present study and seasonal grassland harvest (for hay production) in the available literature?

## 5.2 Experimental

### 5.2.1 Site description

The investigations were performed over grassland at the Dorinne Terrestrial Observatory (DTO, 50° 18’ 44’’ N and 4° 58’ 07’’ E; 248 m a.s.l.). The climate is temperate maritime, with a mean annual air temperature of 9.4 °C and an average annual rainfall of about 905.2 mm (data from the Yvoir weather station which is about 8 km from the DTO, source: Royal Belgian Meteorological Institute, www.meteo.be). Two side-by-side patches of grassland (10 m x 10 m each, Figure 5.1) with identical vegetative composition were chosen at the middle of the DTO for these investigations. Both patches were enclosed separately by fences made of barbed wire and wooden poles to control grazing. Vegetation inside the patches was (mainly) composed of

four species: *Lolium perenne* L., *Trifolium repens* L., *Taraxacum sp*., and *Ranunculus repens* L.. A more detailed description of the field site, vegetation, soil, and climate is given in Digrado et al. (2017).


Figure 5.1: The two grassland patches in the present investigation in DTO.

### 5.2.2 Instrumentation

BVOC, $CO_2$ and $H_2O$ vapour mole fractions in the sampled air were measured using automated dynamic flow-through enclosures (see § 2.2.1), a quadrupole-based hs-PTR-MS (Proton Transfer Reaction–Mass Spectrometer, Ionicon Analytik G.m.b.H., Innsbruck, Austria) and a LI-840A non-dispersive infrared gas analyzer (LI-COR, Lincoln, Nebraska, USA). The PTR-MS technique has been elaborately described in the literature (Hansel et al., 1995; Lindinger et al., 1998) and will therefore not be further discussed here. The PTR-MS instrument was operated under the following conditions: a drift tube pressure of 2.1 hPa, a drift tube temperature of 60 °C and a drift tube voltage of 600 V, resulting in an electric field strength to gas number density ratio (E/N) of 130 Td (1 Td = $10^{-17}$ V $cm^2$). Moreover, the inlet line of the PTR-MS was kept at 60 °C. PTR-MS measurements were carried out in the Multiple Ion Detection (MID) mode, in which the instrument continuously cycled through a list of preset m/z values (will be discussed in section 5.2.5). Gas calibrations for the PTR-MS were performed every 3 days by using a dilute mixture of a set of BVOCs in nitrogen (Apel-Riemer Environmental Inc., Miami, FL, USA). The instrument calibration procedure was described in more detail in Mozaffar et al. (2017).

In addition to quantifying BVOCs by PTR-MS, one sampling for GC-MS analysis was also performed from one of the grazed enclosure for each campaign to assist identification of the emitted BVOCs.

### 5.2.3 Field experiments and sampling procedure

During the growth season of 2016, 3 campaigns with an average duration of ca. 15 days took place between 16 August and 27 September. During each of the investigations, the fences of one of the patches were removed totally to let the cows enter inside the patch and graze. After grazing, which took about 1 hour, the fences were closed again and 3 automated dynamic flow-through enclosures with ancillary equipment (described in § 2.2.1) were installed to perform flux measurements. Another 3 automated dynamic flow-through enclosures with ancillary equipment were installed on the other (undisturbed) patch beforehand. After the full installation, BVOC fluxes from all six cuvettes were alternately monitored for ca. 15 days. After this period the same procedure was performed again, but this time the non-grazed patch in the previous investigation was grazed. We assumed that within those 15 days, grassland would be recovered from the stress of grazing and could be used as a controlled grassland for the next campaign. Indeed, it was the case and the proof of the assumption can be found in § 5.3.3. The alternation of the patches for grazing was followed throughout the investigations. This was done for two main reasons: (i) to increase the number of field campaigns during the growing season and (ii) to increase the reproducibility of the investigated results.

Air was sampled consecutively from enclosures in the grazed and the non-grazed patches according to the scheme presented in Figure 5.2. When air was not being sampled from an enclosure, its lid was kept open in order to expose the enclosed grassland as much as possible to ambient environmental conditions (temperature, rainfall, relative humidity,…). In order to achieve equilibrium conditions in the inflowing airline and in the enclosure before the start of sampling ($t_0$), the axial fan started rotating at $t_0 - \Delta t_1$ and the lid was closed at $t_0 - \Delta t_2$, with $\Delta t_1$ and $\Delta t_2$ being 180 and 55 s, respectively. The total sampling time for one enclosure was 15 minutes, resulting in a time resolution of 1.5 hours when operating the 6 cuvettes sequentially. Of those 15 minutes, incoming ambient air was sampled during the first ($\Delta t_4$) and the last ($\Delta t_6$) four minutes and enclosure air was sampled during the middle 7 minutes ($\Delta t_5$). Finally the lid was opened again 30 seconds ($\Delta t_7$) after the end of the sampling period. Every 3 hours a 15 min sampling period from an enclosure was replaced by a PTR-MS zero measurement which was required for quantifying BVOC mole fractions.

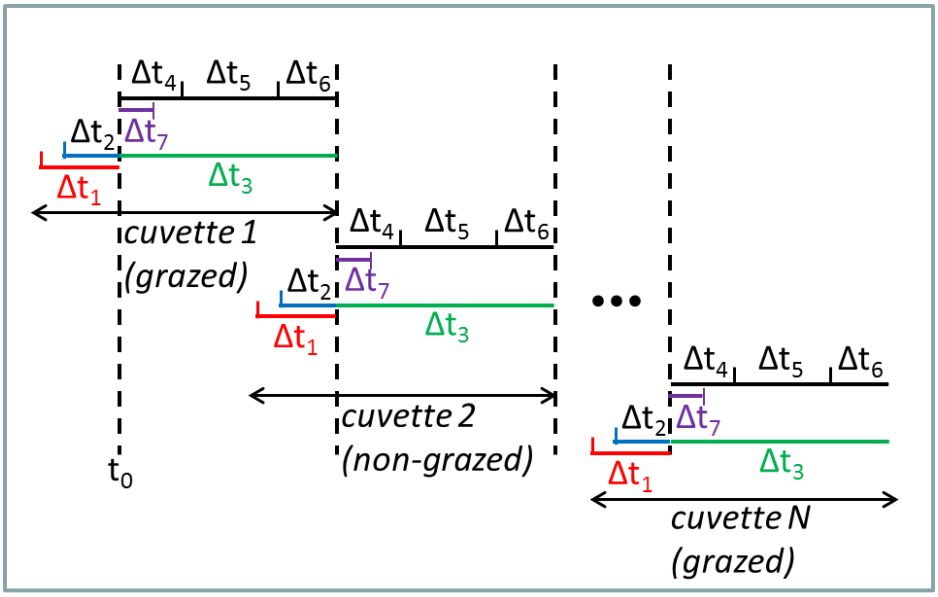



Figure 5.2: Time sequence for subsequent sampling from dynamic enclosure systems. More details can be found in the text.

### 5.2.4 Grass height in the enclosures

The grass height in the undisturbed and grazed enclosures was measured at the beginning (just after the grazing event) and at the end of each campaign using a measuring scale (Table 5.1). The decrease in grass height due to grazing can be estimated from the data in the red and blue rectangles in Table 5.1 for campaign 2 and 3, respectively. Although the height of the undisturbed grassland in enclosures 4, 5 and 6 (1, 2 and 3) in the first (second) campaign was measured a few days before being grazed at the start of the second (third) campaign instead of immediately before the grazing event, it can still be considered as a good approximation of the initial grass height in the grazed enclosure. Data for the initial grass height of the first campaign are lacking. The amount of biomass consumed by the cows, however, could not be estimated from these differences in grass height values by lack of a direct relationship between grass height and leaf biomass.

Table 5.1: Average grass height (cm) in the enclosures measured at the beginning and at the end of each campaign. The grass heights in the enclosures of the grazed grassland patch are shown in italic font. See text for further details.

| | Average grass height (cm) | | | | | |
|---|---|---|---|---|---|---|
| | Campaign 1 | | Campaign 2 | | Campaign 3 | |
| | Start | End | Start | End | Start | End |
| Enclosures | 16/08/2016 | 29/08/2016 | 31/08/2016 | 09/09/2016 | 13/09/2016 | 23/09/2016 |
| 1 | *5* | *13* | 14 | 15 | *4* | *11* |
| 2 | *4* | *14* | 15 | 16 | *2* | *10* |
| 3 | *3* | *12* | 12 | 13 | *2* | *9* |
| 4 | 14 | 18 | *7* | *15* | 16 | 21 |
| 5 | 12 | 17 | *6* | *13* | 14 | 15 |
| 6 | 11 | 17 | *7* | *10* | 10 | 11 |

### 5.2.5 Identification and quantification of emitted BVOCs

During the experiments 20 ion species (including $H_3{}^{18}O^+$, $H_5{}^{18}O^{16}O^+$, i.e. isotopes of the primary ions) were followed in total, resulting in a total cycle time of 40 s. These ion species were chosen based on PTR-MS spectra taken from manually cut grass and also on information in literature on BVOC emissions from grassland. The 18 BVOC-related ion species and their tentative identification are shown in Table 5.2. This identification was mainly based on literature data on emissions from grassland by GC-(FID)-PTR-MS (Davison et al., 2008) and on exact m/z values of compounds exchanges between undisturbed and managed grassland and the atmosphere and compounds emitted from injured vegetation as measured by PTR-TOF-MS (Brilli et al., 2012; Bamberger et al., 2010; Ruuskanen et al., 2011) and also on GC-MS investigations in the present study (Table 5.2). The compounds that could be quantified are indicated in bold and quantification relies upon the assumption that the different compounds contributing to the ion signal at a given m/z value possess similar calibration factors. BVOC mole fractions were obtained by dividing the normalized PTR-MS BVOC ion signals $I_{m/z}$ [ncps] by the experimentally determined BVOC calibration coefficients $C_{BVOC}$ [ncps $ppbv^{-1}$] after substraction of the corresponding normalized ion signals in BVOC-scrubbed air (Mozaffar et al., 2017).

As the calibration bottle used in the present experiments did not contain monoterpenes, the monoterpenes calibration factors $C_{MT,81}$ and $C_{MT,137}$ obtained during previous long-term

calibrations of the PTR-MS with a calibration mixture containing monoterpenes (452 ppbv alpha-pinene and 399 ppbv sabinene) have been used to calculate monoterpenes concentrations. Unfortunately the ion species at m/z 137 was only followed during the last of the three experiments. The net normalized count rates for m/z 137 for undisturbed grassland followed very well those for m/z 81. This was also the case for the grazed parcels except for a day and a half after the grazing event. This suggests that the m/z 81 compounds which are emitted from undisturbed grassland are monoterpenes, whereas shortly after the grazing they comprise both hexenals (mainly for the first one and a half day) and monoterpenes. A similar conclusion was also made by Ruuskanen et al. (2011) for their investigations on grassland experiments. For the current investigations we will assume that the ion signal at m/z 81 from the undisturbed grassland was totally due to monoterpenes and for the grazed grassland, one and a half day after the grazing event it was totally due to hexenals.

The sum of ion signals at m/z 57, 81 and 99, in combination with a lumped calibration coefficient, was used to infer the concentration of the sum of hexenals, as already explained in chapter 4, Section 4.2.3.

Table 5.2: Mass-to-charge values of ion signals measured during the grassland campaigns and their tentative identification based on literature data. Fluxes for compounds with m/z values given in bold have been quantified with the calibration coefficients of the corresponding compounds given in bold font. The dwell time for each of the measured ion signals was 2 s.

| **nominal m/z** | **Molecular formula** | **Literature-based tentative identification of the associated compound(s)** |
|---|---|---|
| **33** | $CH_3OH$-$H^+$ | **methanol** [a,b,c1,c2,c3,d] |
| 43 | | hexyl acetate [a], hexanol [a], acetic acid [c1], methylbutanals+pentenols [c1], methyl acetate [d] |
| **45** | $C_2H_3OH$-$H^+$ | **acetaldehyde** [a,b,c1,c3,d], 2-pentanone (fragment) [d], 1-penten-3-ol [d] |
| 57 | $C_4H_8$-$H^+$ | hexenals [†,a,c1,d], butanol [d] |
| **59** | $C_3H_6O$-$H^+$ | **acetone** [b,c1,d], propanal, pentanol [d] |
| **61** | $C_2H_4O_2$-$H^+$ | **acetic acid** [b,c1,c2,c3], hexyl acetate [a] |
| 69 | $C_5H_8$-$H^+$ | isoprene [a,c1], pentenols [a,c1,d], methylbutenols, methylbutanals [c1] |

| | | |
|---|---|---|
| **71** | $C_4H_6O$-$H^+$, $C_5H_{11}^+$ | **methyl vinyl ketone** [a,b,c1,c3,], **methacrolein** [a,b,c1,c3], pentanol [a,d] |
| **73** | $C_4H_8O$-$H^+$ | **(2-)butanone** [b,c1,c3,d], butanal [c1,c3,d] |
| **81** | $C_6H_8$-$H^+$ | **hexenals** [†, a,c1,c3,d], **monoterpenes**[†,c3] |
| **83** | $C_6H_{10}$-$H^+$ | **hexenols**[†], hexanal[†], hexenyl acetates [†,a,c1,c3,d] |
| 85 | $C_5H_8O$-$H^+$, $C_6H_{12}$-$H^+$ | pentenone [a,b], hexanol [†,a,b], pentenal |
| 87 | $C_5H_{10}O$-$H^+$ | methylbutanals, pentenols [†,b,c1,c3], 2-pentanone [d] |
| 99 | $C_6H_{10}O$-$H^+$ | hexenals [†,a,b,c1,c3,d], 3-methyl-penten-2-one [d] |
| 101 | $C_6H_{12}O$-$H^+$ | hexenols[†], hexanal [†,a,b,d] |
| 115 | $C_7H_{14}O$-$H^+$ | Heptanal [d] |
| 129 | $C_8H_{16}O$-$H^+$ | Octanal [d], 2-ethylhexanol [d] |
| 137* | $C_{10}H_{16}$-$H^+$ | **monoterpenes** [†,a,b,c1,c2,c3] |
| 143 | $C_8H_{14}O_2$-$H^+$ | hexenyl acetates [†,a,b] |

[a] Brilli et al. (2011) – PTR-TOF-MS
[b] Brilli et al. (2012) – PTR-TOF-MS
[c] Ruuskanen et al. (2011) – PTR-TOF-MS – c1: VOCs exchanged with grassland before, during and after harvesting – c2: VOCs exchanges with intact grass – c3: VOCs exchanged over drying, cut grass
[d] Davison et al. (2008) – PTR-Quad-MS
* This ion species was only followed during one of the campaigns.
† compounds that were detected by GC-MS. Identified monoterpenes were D-limonene and ocimene

### 5.2.6 Quality control and flux calculation

Fluxes (F) of BVOCs, $H_2O$ (evapotranspiration) and $CO_2$ (net photosynthesis) from the enclosed grassland were all expressed as molar fluxes per unit of soil surface area [mol $m_{soil}^{-2}$ $s^{-1}$] and were calculated with the following formula:

$$F = \frac{F_{air}(X_{PE} - X_{IN})}{A} \quad \text{(Eq. 1)}$$

in which $F_{air}$ is the molar flow rate of ingoing ambient air, $X_{PE}$ and $X_{IN}$ are the mole fractions (of BVOCs, $H_2O$ or $CO_2$) in the plant enclosure and the ingoing ambient air, respectively, and A is the enclosed soil surface area (0.096 $m^2$).

Additionally, two filtering steps were applied to handle potential sampling artefacts.

First, when sampling air from one of the six enclosures, the sampling lines of the other 5 enclosures were continuously purged by a small air flow. Nevertheless, sharp BVOC transient ion signals sometimes occurred when switching between sampling lines in the manifold and these were not taken into account by leaving out the first 120 s of measured data when computing $X_{PE}$ and $X_{IN}$.

Second, as mentioned in § 5.2.3, inflowing ambient air was sampled both before and after sampling enclosure air. For all compounds the average of the incoming air mole fractions, ($x_{IN1}$ + $x_{IN2}$)/2, was taken into account for further analysis. However, events showing large fluctuations in incoming air concentration would end in spiky and unrealistic fluxes (under/overestimations). Consequently, a filtering criterion was applied to remove such situations. Data were not considered when the net absolute concentration difference ( $\left| x_{PE} - (x_{IN1} + x_{IN2})/2 \right|$ ) was smaller than twice the maximum of the absolute difference between the two inflowing air concentrations ( $\left| x_{IN1} - x_{IN2} \right|$ ) and the standard deviations on the inflowing air concentrations measurements (Eq. 2).

$$\left| X_{PE} - \frac{1}{2}(X_{IN1} + X_{IN2}) \right| \leq 2 \times max\left( \left| X_{IN1} - X_{,IN2} \right|, \sigma_{X_{IN1}}, \sigma_{X_{IN2}} \right) \qquad \text{(Eq. 2)}$$

Moreover, in order to avoid elimination of all small BVOC, $H_2O$ and $CO_2$ flux values with this criterion, data rejection was withheld if the net absolute concentration difference ( $\left| x_{PE} - (x_{IN1} + x_{IN2})/2 \right|$ ) was less than 5% of its maximum value over the entire measurement period. After applying these filtering criteria, 1-4% of the fluxes were removed of m/z 33, m/z 73, m/z 81 compounds and $H_2O$ and 12-40% of the fluxes of compounds associated with the remaining m/z ratios and with $CO_2$.

When measured data met the second filtering criterion, the ambient air concentration $X_{IN}$ of BVOC, $H_2O$ and $CO_2$ during the 7 minutes period of enclosure air sampling was assumed to be equal to the average value of the two inflowing air concentrations, i.e. ($X_{IN1}$+$X_{IN2}$)/2.

### 5.2.7 Statistical analysis

Two-way ANOVA and Spearman correlation analysis were performed with the Matlab® R2013a statistics toolbox (The MathWorks, Inc., Natick, Massachusetts, USA).

## 5.3 Results and discussion

### 5.3.1 Constitutive BVOC fluxes

Among the quantified BVOCs from the undisturbed grassland, methanol (m33) and butanone/butanal (m73) were emitted during daytime (Fig. 5.3). At night, flux values were close to zero. Besides, acetone (m59) and monoterpenes (m81) were deposited from late afternoon to early morning and emitted during the rest of the day. On the other hand, acetaldehyde (m45), acetic acid (m61), methyl vinyl ketone/methacrolein (m71) and hexenols/hexenyl acetates (m83) were deposited all the time. Methanol followed by acetic acid and acetaldehyde were the BVOCs with the highest exchange rates whereas hexenols/hexenyl acetates were the least exchanged compounds. The maximum flux intensity of the exchanged compounds was observed during midday (except for acetic acid, for which no clear trend was observed) and the minimum during the night.

Among the constitutively emitted compounds, methanol is known to be produced in leaves as a result of changes in the cell wall structure during growth (Fall, 2003; Huve et al., 2007). Given an average increase in grass height of 0.31±0.17 cm $day^{-1}$ for the undisturbed grassland, vegetation is expected to be the main contributor to methanol fluxes from the grassland ecosystem. However, contributions from soil cannot a priori be excluded (Schade and Custer, 2004). Emissions of acetone and butanone/butanal from undisturbed grass and other plant species have been reported previously (Amman et al., 2007; Bracho-Nunez et al., 2013; Cappellin et al., 2017) but their production mechanisms have not yet been elucidated. Similar as for methanol, soil has also been reported as a potential source of those compounds (Fall, 2003; Schade and Custer, 2004; Asensio et al., 2008) and should therefore not be discarded in our dynamic grassland enclosure measurements. Monoterpenes can be constitutively emitted by plants as a defence mechanism against herbivores and pathogens (Gershenzon et al., 2000) and to attract pollinators (Pichersky and Gershenzon, 2002), and consequently monoterpene emission from grassland ecosystems is not irrational. Uptake of acetaldehyde, acetic acid, methyl vinyl ketone/methacrolein and hexenols/hexenyl acetates to the grassland ecosystem can take place by diffusional transport through the stomata (Tani and Hewitt, 2009) or by adsorption to the leaf (Tani and Hewitt, 2009; Sugimoto et al., 2016) or soil surfaces (Spielmann et al., 2017). In case of stomatal uptake, the BVOC deposition rate will depend on stomatal conductance and the

BVOC concentration gradient between ambient air and the substomatal cavity. After entering the leaf, the BVOCs may be metabolised and/or translocated through the leaf petiole (Tani and Hewitt, 2009).

Except for methanol, information about BVOC fluxes from undisturbed grassland is scarce and available data from the literature are gathered in Table 5.3. Overall large variations were observed among the available studies and our observations are at the lower end of the flux ranges. The magnitude of acetone emission in the present study was for instance found to be 50-25% of those reported by Warneke et al. (2002) and Amman et al. (2007), respectively, and our maximum butanone/butanal flux value is 25-10% of the one mentioned by Amman et al. (2007). Moreover, not only flux magnitudes but also flux directions varied among studies for some BVOCs. While depositions were noticed for acetaldehyde in this study, Warneke et al. (2002) and Amman et al. (2007) reported emissions. In addition, monoterpene depositions were noticed by Ruuskanen et al. (2011) in contrast to the observed emissions in the current investigation. By contrast, the acetic acid maximum flux value in our study is very similar to the one reported by Ruuskanen et al. (2011). Several factors may contribute to the large variations in BVOC flux intensities from undisturbed grasslands among studies. First, strong differences in grassland species compositions have been reported for the grassland measurement sites reported in Table 5.3 and previous studies have already revealed large quantitative differences in BVOC fluxes from different grass species (e.g. Brilli et al., 2012). Second, BVOC fluxes in the different studies have not been normalized to standard environmental conditions and are therefore influenced by local meteorological conditions. Third, local ambient BVOC concentration and weather conditions also control the direction (emission/deposition) of the fluxes (Karl et al., 2010; Seco et al., 2007). The large monoterpene deposition rates that were reported by Ruuskanen et al. (2011) were for instance related to high ambient monoterpene concentrations due to strong releases by conifers after a hail storm. Finally, soil type, soil microorganism composition and type of fertilizer/slurry used or organic matter and microorganisms brought in by fertilization may have been different at the different measurement sites and this may also have influenced BVOC flux spectra and intensities (Ramirez et al., 2010; Brilli et al., 2012).

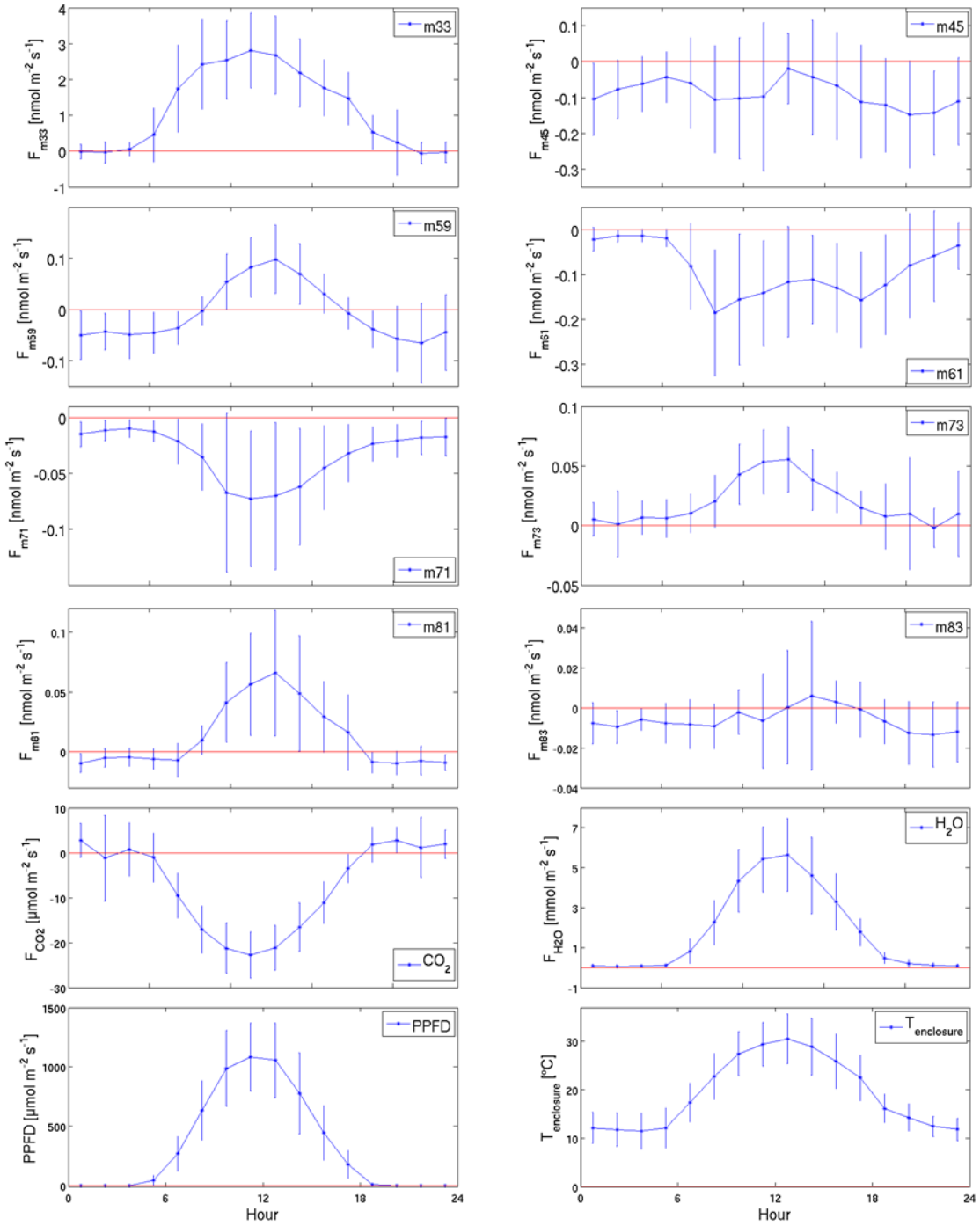



Figure 5.3: Average daily fluxes of BVOC, $H_2O$ and $CO_2$ from undisturbed grassland with PPFD and temperature conditions. These average fluxes were estimated over results from 3 campaigns, each on 3 enclosures. The error bars correspond to the standard deviation on the measurements over 1.5 hours bins. Here m33 is methanol, m45 is acetaldehyde, m59 is acetone, m61 is acetic acid, m71 is methyl vinyl

ketone/methacrolein, m73 is butanone/butanal, m81 is monoterpenes and m83 is hexenols/hexenyl acetates.

Table 5.3: Approximate maximum BVOC flux [nmol $m^{-2}$ $s^{-1}$] from undisturbed grassland with corresponding study site and method used. Flux values are averaged over 1.5 hours of measurement for the current study, over 1 hour for the Brunner et al. (2007) study and over 0.5 hours for the investigations of Warneke et al. (2002), Bamberger et al. (2010), Hörtnagl et al. (2011) and Ruuskanen et al. (2011). Investigations of Amman et al. (2007) and Brunner et al. (2007) were performed on the same grassland site in Switzerland. The studies of Bamberger et al. (2010), Hörtnagl et al. (2011) and Ruuskanen et al. (2011) were all conducted on the same grassland site in Austria.

| | Study site | method | methanol | acetaldehyde | acetone | acetic acid | methyl vinyl ketone/methacrolein | butanone/butanal | monoterpenes | hexenols/hexenyl acetates |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | m/z 33 | m/z 45 | m/z 59 | m/z 61 | m/z 71 | m/z 73 | m/z 81 | m/z 83 |
| Current study | agricultural grassland, Belgium | chamber, PTR-MS | 3 | -0.15 | 0.1 | -0.2 | -0.07 | 0.05 | 0.15 | ~0 |
| Warneke et al. (2002) | alfalfa field, USA | DES, PTR-MS | 32.9 | 0.4 | 0.2 | | | | | |
| Amman et al. (2007) | agricultural grassland, Switzerland | chamber, GC-FID-PTR-MS | 3-8 | 0.5-0.8 | 0.4 | | | 0.2-0.7 | | |
| Brunner et al. (2007) | agricultural grassland, Switzerland | EC, PTR-MS | 4-10 | | | | | | | |
| Bamberger et al. (2010) | agricultural grassland, Austria | EC, PTR-MS | 6 | | | | | | | |
| Hörtnagl et al. (2011) | agricultural grassland, Austria | EC, PTR-MS | 2-10 | | | | | | | |
| Ruuskanen et al. (2011) | agricultural grassland, Austria | EC, PTR-TOF-MS | 9.31 | | | -0.19 | | | -8.21 | |

## 5.3.2 Controls on constitutive BVOC fluxes

A high positive correlation between fluxes of methanol, acetone, butanone/butanal, monoterpenes and PPFD, temperature, evapotranspiration and net photosynthesis rates was observed in the present study (|ρ|=0.43 to 0.76), (Fig. 5.4, Table 5.4). An increase of these compound fluxes with increasing temperature can be explained by enhanced rates of the enzymatic reactions involved in BVOC production and enhanced partitioning of these compounds in the gas-phase (Niinemets and Reichstein, 2003a, 2003b) in the biomass/leaf interior. The positive correlation between the emission rates of the oxygenated water soluble compounds and transpiration rates under high light conditions is also reasonable as light stimulates stomatal opening and brings out these compounds to the atmosphere with increased water loss. Positive correlations between light conditions, temperature and fluxes of methanol, acetone, butanone/butanal and monoterpenes from grassland and also from other vegetation have already been mentioned by several other authors (Brunner et al., 2007; Kistine et al., 1998; Hortnagl et al., 2011, Harley et al., 2007; Filella et al., 2007; Min and Wildt, 2002; Jardine et al., 2015; Loreto et al., 2006).

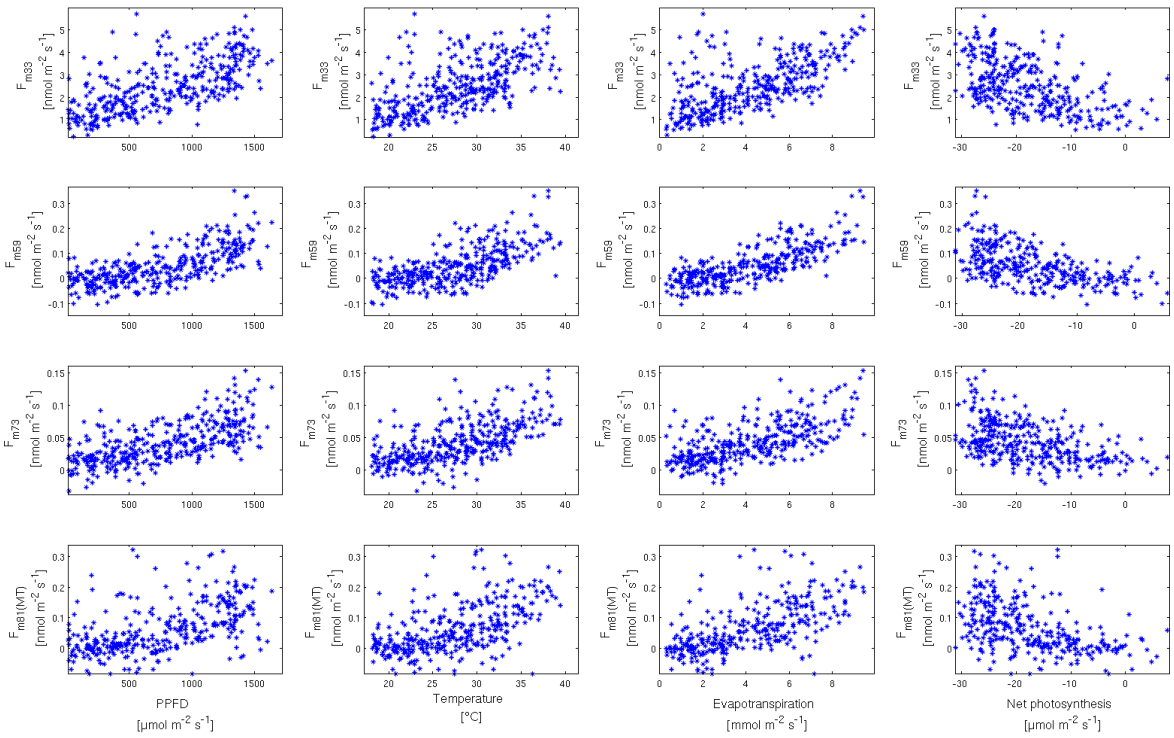


Figure 5.4: Methanol (m33), acetone (m59), butanone/butanal (m73) and monoterpenes (m81) fluxes as a function of PPFD, temperature, evapotranspiration and net photosynthesis.

Fluxes of acetaldehyde, acetic acid, methyl vinyl ketone/methacrolein and hexenols/hexenyl acetates, for which depositions were observed, were negatively correlated ($|\rho|$=0.39 to 0.93) with the ambient concentrations of the corresponding compounds (Fig. 5.5, Table 5.4). Those concentrations were probably influenced by emissions from surrounding vegetation or farming activities. The correlation with ambient concentration was particularly good for methyl vinyl ketone/methacrolein ($|\rho|$=0.93), the only deposited compounds for which a reasonably good correlation was found with PPFD, air temperature, evapotranspiration and net photosynthesis rate as well. As the latter parameters are all strongly related to stomatal conductance and since only small depositions were observed during the night, methyl vinyl ketone/methacrolein is probably mainly taken up by the leaves through the stomata. Increase of acetaldehyde uptake with increasing ambient concentration was also mentioned by Kesselmeier (2001), Rottenberger et al. (2004) and Karl et al. (2005) for *Quercus ilex*, 3 Amazonian plant species and *Pinus taeda*, respectively. Moreover, enhanced deposition of acetic acid and methyl vinyl ketone with increasing ambient concentration was also observed by Jardine et al. (2011) in the Amazonian rain forest and by Fares et al. (2015) and Cappellin et al. (2017) for 3 different *Quercus* species and red oaks, respectively. A better correlation between BVOC fluxes and net photosynthesis rates was found for all the daytime emitted compounds compared to the other compounds (see supplementary section S5.1). The reason for this observed correlation is probably due to the inter-correlation between good weather conditions (high light and temperature), high $CO_2$ uptake and high constitutive emissions. Relative to the other parameters, relative humidity showed poor correlation with the fluxes. Further analysis is clearly needed to elucidate the controlling factors of BVOC fluxes from the undisturbed grassland.

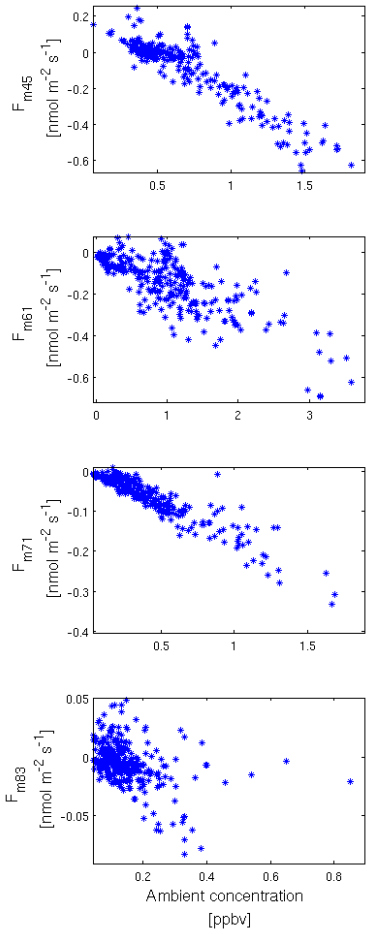


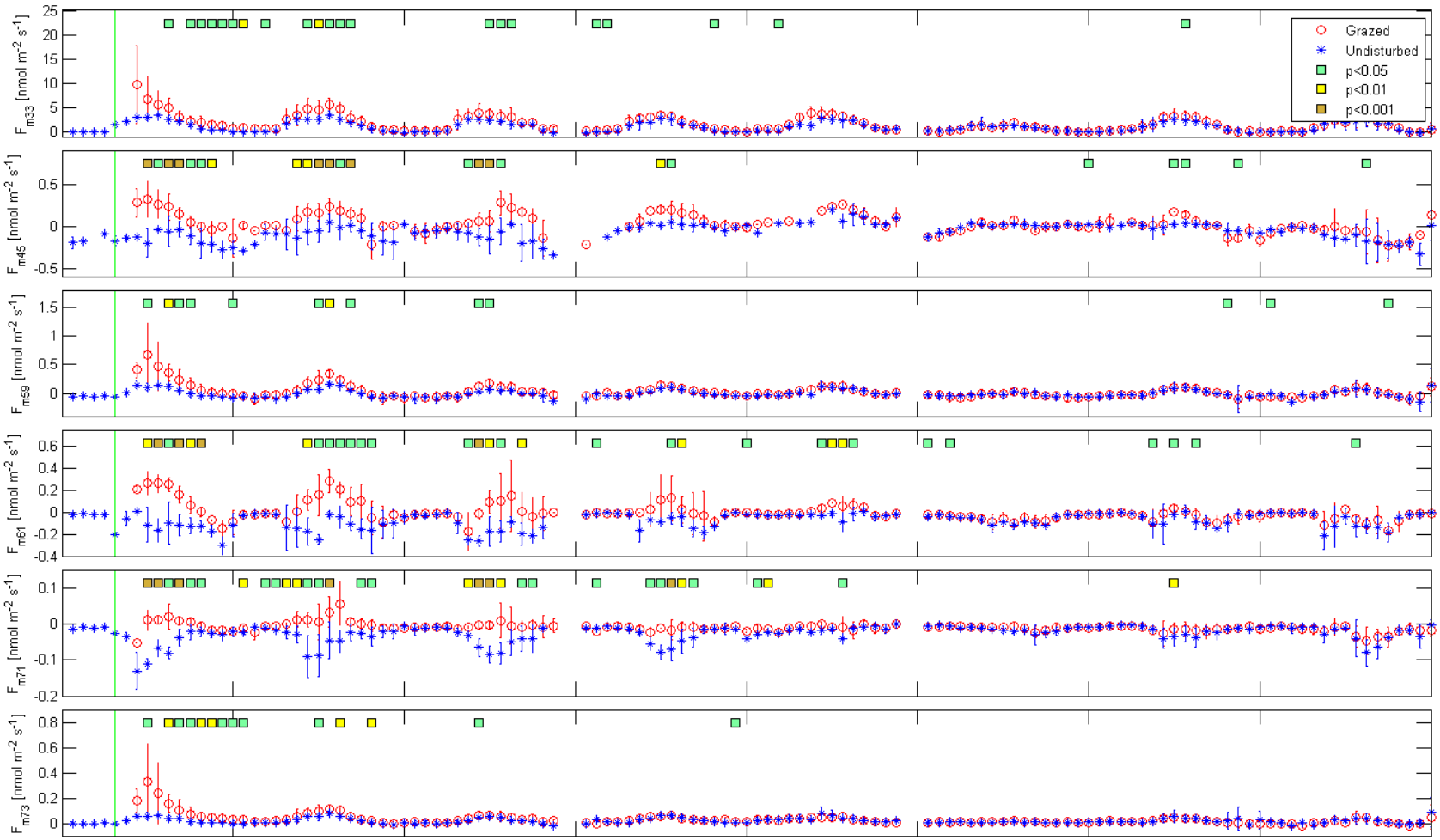

Figure 5.5: Acetaldehyde (m45), acetic acid (m61), methyl vinyl ketone/methacrolein (m71) and hexenols/hexenyl acetates (m83) fluxes as a function of ambient condition.

Table 5.4: Spearman's correlation coefficient (rho) values obtained between one and half hourly BVOC fluxes from undisturbed grassland and different environmental parameters and fluxes of $CO_2$ and $H_2O$. (*:p<0.05; **:p<0.01; ***:p<0.001 and ns:not significant.). Here m33 is methanol, m45 is acetaldehyde, m59 is acetone, m61 is acetic acid, m71 is methyl vinyl ketone/methacrolein, m73 is butanone/butanal, m81 is monoterpenes and m83 is hexenols/hexenyl acetates.

| Compounds | PPFD | Temperature | Evapotranspiration | Net photosynthesis | Ambient concentration | Relative humidity |
|---|---|---|---|---|---|---|
| | | | Spearman's correlation coefficient (rho) | | | |
| m33 | 0.67 (***) | 0.56 (***) | 0.60 (***) | -0.52 (***) | 0.36 (***) | -0.16 (***) |
| m45 | -0.07 (ns) | -0.30 (***) | -0.15 (**) | -0.01 (ns) | -0.79 (***) | -0.01 (ns) |
| m59 | 0.71 (***) | 0.66 (***) | 0.76 (***) | -0.52 (***) | 0.03 (ns) | -0.47 (***) |
| m61 | 0.16 (**) | -0.11 (ns) | 0.02 (ns) | -0.08 (ns) | -0.69 (***) | -0.11 (*) |
| m71 | -0.39 (***) | -0.66 (***) | -0.55 (***) | 0.28 (***) | -0.93 (***) | 0.25 (***) |
| m73 | 0.66 (***) | 0.63 (***) | 0.65 (***) | -0.43 (***) | 0.21 (***) | -0.22 (***) |
| m81 | 0.55 (***) | 0.59 (***) | 0.65 (***) | -0.44 (***) | -0.28 (***) | -0.44 (***) |
| m83 | -0.08 (ns) | -0.11 (*) | -0.06 (ns) | -0.03 (ns) | -0.39 (***) | -0.14 (**) |

### 5.3.3 Grazing-induced BVOC fluxes and their comparison with the constitutive fluxes

Grazing resulted in enhanced emissions of BVOC compounds which lasted for 2-5 days including the grazing day (Fig. 5.6). ANOVA tests confirmed that the differences between fluxes from the grazed and undisturbed grassland patches were statistically significant. While daytime depositions of acetaldehyde, acetic acid and methyl vinyl ketone/methacrolein were noticed from the undisturbed grassland, emissions were observed from the grazed grassland. Similar to the undisturbed grassland, methanol was the highest exchanged compound from the grazed grassland. In addition, the maximum flux intensity of the exchanged compounds was observed during midday and the minimum flux intensity during the night.


Grazed
Undisturbed
p<0.05
p<0.01
p<0.001
F_m33 [nmol m^-2 s^-1]
F_m45 [nmol m^-2 s^-1]
F_m59 [nmol m^-2 s^-1]
F_m61 [nmol m^-2 s^-1]
F_m71 [nmol m^-2 s^-1]
F_m73 [nmol m^-2 s^-1]

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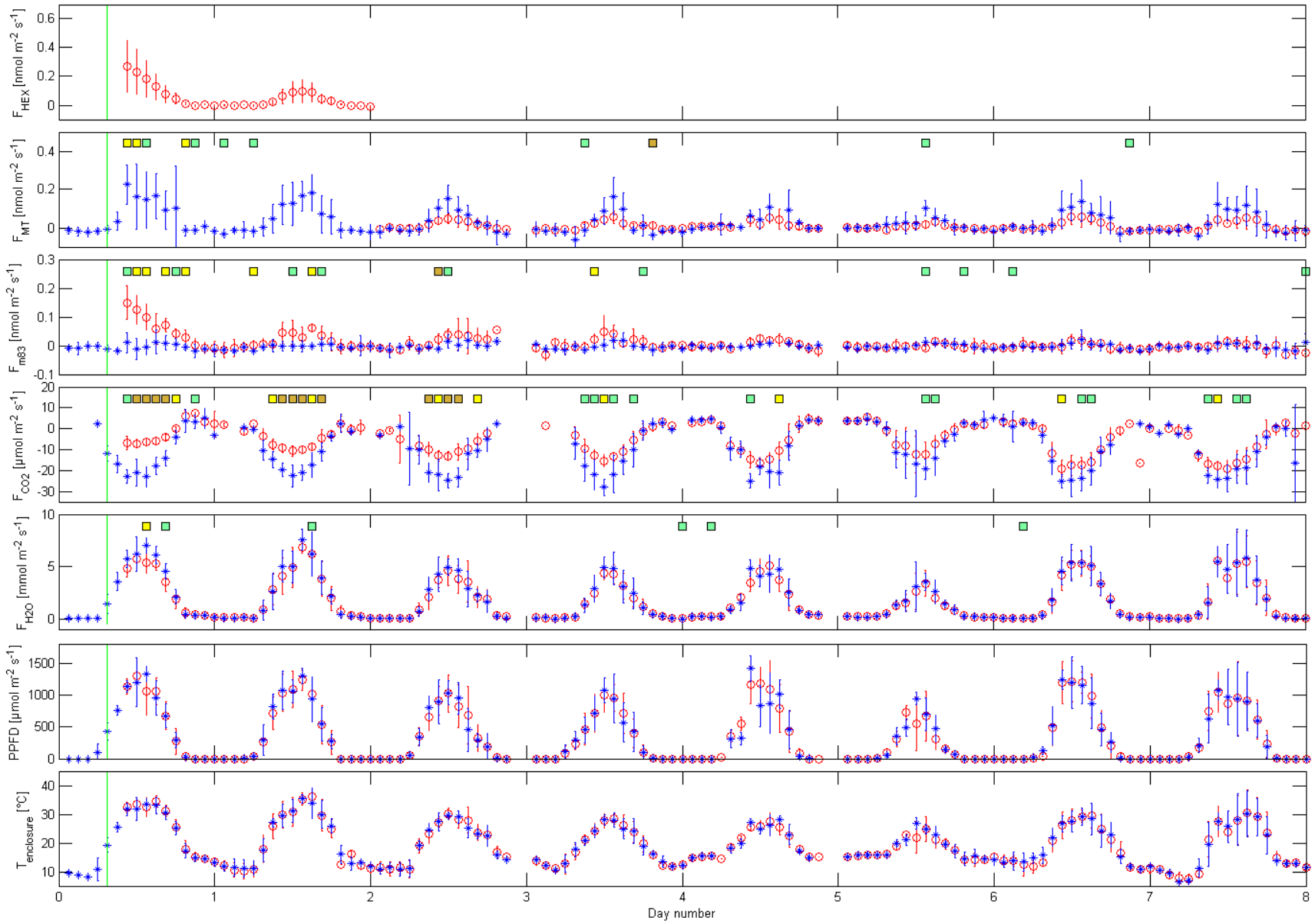
F_HEX [nmol m-2 s-1]
F_MT [nmol m-2 s-1]
F_m83 [nmol m-2 s-1]
F_CO2 [µmol m-2 s-1]
F_H2O [mmol m-2 s-1]
PPFD [µmol m-2 s-1]
T_enclosure [°C]
Day number

Figure 5.6: Fluxes of BVOC, $H_2O$ and $CO_2$ from grazed and undisturbed grassland with PPFD and temperature conditions. The error bars correspond to the standard deviation on the measurements over 1.5 hours bins. These fluxes are obtained from the results of 3 campaigns, and a set of 3 enclosures were used in each campaign both on the undisturbed and grazed grassland. The green, yellow and gold squares above the flux values indicate the level of significance of the differences between the fluxes from the grazed and undisturbed grassland using ANOVA statistics. The green vertical line indicates the end of the grazing event and after this line, the missing data points from the grazed grassland is due to the time taken for the installation of the enclosures. Here m33 is methanol, m45 is acetaldehyde, m59 is acetone, m61 is acetic acid, m71 is methyl vinyl ketone/methacrolein, m73 is butanone/butanal, MT is monoterpenes, HEX is hexenals and m83 is hexenols/hexenyl acetates.

For the grazed grassland, the BVOCs with the highest 7.5-days-accumulated fluxes are methanol, butanone/butanal, acetone and acetaldehyde (Fig. 5.7). The emissions of methanol and butanone/butanal after the grazing increased by 57 and 62%, respectively, compared to those from the undisturbed patch over the same accumulation period. While 36, 4, and 1 µmol $m^{-2}$ of acetaldehyde, acetone and hexenols/hexenyl acetates, respectively, were deposited on the undisturbed patch, emissions of 15, 20, 6 µmol $m^{-2}$ were observed from the grazed patch over the accumulation period. As the direction of the fluxes was different for the grazed and undisturbed patches, the relative reduction or increase of the fluxes cannot be estimated for these 3 compounds. In addition, deposition of acetic acid and methyl vinyl ketone/methacrolein decreased by 97 and 64% on the grazed patch compared to the undisturbed patch over the accumulation period.

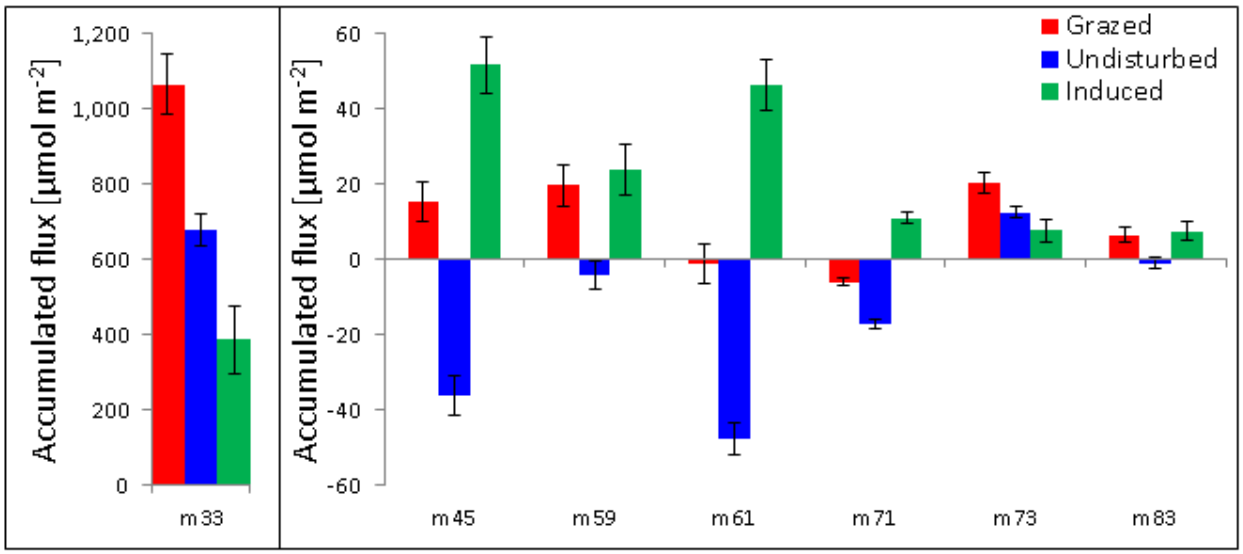


Figure 5.7: Accumulated BVOC fluxes from the grazed and undisturbed grassland measured over 7.5 days and their differences (induced emissions). The accumulated induced flux is the difference between the accumulated BVOC flux from grazed and undisturbed grassland. Here m33 is methanol, m45 is acetaldehyde, m59 is acetone, m61 is acetic acid, m71 is methyl vinyl ketone/methacrolein, m73 is butanone/butanal and m83 is hexenols/hexenyl acetates.

The differences in BVOC flux between the undisturbed and grazed grassland can be explained by the biotic injury of the biomass and subsequent production and emission of stress compounds (Fall et al., 1999; Niinemets et al., 2013). Indeed, previous studies have demonstrated that methanol emission can be enhanced by wounding and herbivore damage due to increased production rates (Hann et al., 2014; Loreto et al., 2006) and that, upon wounding, fatty acid oxidation by reactive oxygen species (ROS) enhances acetaldehyde production (Jardine et al., 2009). After injury, induced acetaldehyde emissions from other plants were also noticed by Brilli et al., 2011. Furthermore, cyanogenic plants are known to produce acetone after damage or injury (Fall, 2003). Among the four plant species in the enclosures, *Trifolium repens* L. and *Taraxacum* sp. are cyanogenic plants (Francisco and Pinotti, 2000; Ballhorn and Elias, 2014) and acetone emissions from the grazed grassland could therefore be expected. Besides, after wounding, when fatty acids are oxidized by ROS, high levels of acetyl-CoA are generated, and hydrolysis of acetyl-CoA could lead to the formation of acetic acid (Fall, 1999). So, it is quite

understandable to notice high acetic acid emissions from the grazed grassland. Hexenals, hexenols and hexenyl acetates are stress-induced compounds from plants and are produced from fatty acid oxidation via the lipoxygenase (LOX) pathway (Scala et al., 2013; Dudareva et al., 2013) following physical damage (Fall et al., 1999). According to the literature, after wounding, the initial burst of BVOC emissions lasts for 10 to 40 minutes and emission rates subsequently decrease slowly with time (Fall et al., 1999; Brilli et al., 2011). After grazing, BVOCs probably escaped to the atmosphere both via the injured leaf surface (Brilli et al., 2011) and also via the stomata until the wound was healed. The time it takes the wound to heal due to grazing is not mentioned in the literature yet. After the wound was healed, BVOCs probably escaped only through the stomata from the grazed grassland as the diurnal evolution of the BVOC fluxes followed the diurnal evolution of evapotranspiration and net photosynthesis rates. However, the temperature reduction by around 20 °C at night cannot be ruled out as a possible reason for the nighttime reduction of BVOC fluxes. Other investigators also observed nighttime decrease and daytime increase of BVOC fluxes from cut grasses (Eller et al., 2011; Ruuskanen et al., 2011; Bamberger et al., 2010).

### 5.3.4 Comparison between grazed and harvest-induced BVOC fluxes

Since there is no information in the literature about grazing-induced BVOC fluxes from a grassland, the closest investigations against which the observations obtained in the current study can be compared are the grassland harvest (hay production) experiments reported by Ruuskanen et al. (2011), Davison et al. (2008), Brunner et al. (2007), Bamberger et al. (2010), Hörtnagl et al. (2011), Warneke et al. (2002), Karl et al. (2001a), and Karl et al. (2001b). However, comparison is still not straightforward, considering the differences between the two grassland management practices and the differences in plant species compositions and environmental conditions. Harvest-induced emissions are typically one to two orders of magnitude larger than our grazing-induced emissions (Table 5.5). However, the flux values for the grazed grassland should be considered as lower limits as flux measurements were started about 3 hours after the end of the grazing event. Indeed, Kirstine et al. (1998) observed an exponential decrease of BVOC fluxes from wounded grass and flux rates reduced to one half of the initial value in 2.3 hours (with a decay constant of -0.3 $h^{-1}$ from an initial flux rate of 182 $\mu gCg_{DW}^{-1}h^{-1}$). The observed lower values (Table 5.5) of the initial maximum fluxes in the grazing experiment compared to the harvest experiments could have several reasons apart from missing the burst of emissions just

after the wounding. First, the extent of wounding was possibly lower for the grazed grassland than for the harvested grassland. For the grazing study grasses were severed at around 4 cm height where the leaf density was low and each leaf was severed just once. In contrast, more leaves were probably severed for the harvest experiments due to the machine cut at higher elevation than 4 cm and probably each leaf was severed several times due to continuous rotation of the mowing blades. Indeed, induced flux intensity is proportional to the extent of wounding or injury (Copolovici et al., 2011; Brilli et al., 2011). Second, the initial biomass, over which the fluxes were measured, was much lower in the grazing experiments than in the harvesting experiments, since severed grasses were left on the harvested grassland for drying. The second reason is also applicable for the explanation of the lower accumulated fluxes from the grazed grassland. In addition, drying grasses (on the harvested grassland) are an important source of BVOCs (Karl et al., 2001b; Karl et al., 2005). Harvesting events happen only 3 to 4 times a year (Bamberger et al., 2010) whereas grazing events happen around 6 times a year (personal communication Louis Gourlez De La Motte ) in a rotationally grazed cattle farm in Belgium. Therefore, although relatively higher amounts of BVOCs are emitted after a grassland harvesting event, the annual grazing-induced BVOC fluxes could still be smaller than the harvest-induced fluxes.

Table 5.5: Comparison of BVOC fluxes from grazed and harvested grassland

| Compound | methanol | Acetaldehyde | Acetone | acetic acid | methyl vinyl ketone/methacrolein | butanone/butanal | hexenals | hexenols/hexenyl acetates |
|---|---|---|---|---|---|---|---|---|
| m/z | 33 | 45 | 59 | 61 | 71 | 73 | 81 | 83 |
| Maximum flux value after grazing/harvest [nmol $m^{-2}$ $s^{-1}$] | | | | | | | | |
| Current study | 10 | 0.4 | 0.7 | 0.3 | 0.03 | 0.3 | 0.3 | 0.15 |
| Karl et al. (2001a) | 309-436.9 | 20.4-34.1 | 0.3-1.2 | | | 0.6-1.1 | | |
| Karl et al. (2001b) | 72.8 | 18.9 | 7.2 | | | | | |
| Warneke et al. (2002) | 69.4 | 2.2 | 1.2 | | | | | |
| Brunner et al. (2007) | 30-110.9 | | | | | | | |
| Davison et al. (2008) | 91.5 | 19.4 | 12.7 | | | 5.6 | 8.9 | |
| Bamberger et al. (2010) | 78.4 | 11 | | | | | 8.3 | |
| Hörtnagl et al. (2011) | 28.1-144.5 | | | | | | | |
| Ruuskanen et al. (2011) | 98.1 | 10.7 | 2.2 | 1.96 | 0.39 | 0.7 | 7.55 | 4.8 |
| Average daytime flux over the first 3 days after grazing/harvest [nmol $m^{-2}$ $s^{-1}$] | | | | | | | | |
| Current study | 4.1 | 0.14 | 0.18 | | | 0.09 | 0.11 | 0.04 |
| Davison et al. (2008) | 21.1 | 5.1 | 2.6 | | | 1.1 | 2.6 | 2.3 |
| Cumulative flux over the first 24 hours after grazing/harvest [mmol $m^{-2}$ ] | | | | | | | | |
| Current study | 0.28 | 0.0065 | 0.012 | 0.005 | -0.00047 | 0.008 | 0.0055 | 0.0032 |
| Ruuskanen et al. (2011) | 3.85 | 0.58 | 0.1 | 0.1 | 0.03 | 0.05 | 0.02 | 0.1 |

## 5.4 Conclusions

We provided the first investigations of BVOC fluxes from a grassland due to grazing, which is one of the common biotic stresses on these vast ecosystems. These investigations were performed on a managed grassland in Belgium using automated dynamic flow-through chambers and Proton Transfer Reaction–Mass Spectrometry. The quantified constitutive BVOCs from this study were methanol, butanone/butanal, acetone, monoterpenes, acetaldehyde, acetic acid, methyl vinyl ketone/methacrolein and hexenols/hexenyl acetates. The first two of them were emitted during the day, the third and fourth were deposited from late afternoon to early morning and emitted during the rest of the day, and the last four were deposited on the grassland all the time. Among these compounds, methanol and hexenols/hexenyl acetates were the highest and least exchanged compounds, respectively. Whereas fluxes of methanol, butanone/butanal, acetone and monoterpenes were best correlated with light intensity, temperature, evapotranspiration, and net photosynthesis rates, uptake of acetaldehyde, acetic acid, methyl vinyl ketone/methacrolein and hexenols/hexenyl acetates was best correlated with ambient concentration. Grazing not only significantly increased BVOC fluxes but also altered the fluxes qualitatively (flux direction) with respect to the constitutive ones. Even if grazing-induced BVOC fluxes are higher than the constitutive ones, they are typically one to two orders of magnitude lower than the grassland harvest-induced fluxes (for hay production) mentioned in the literature. The annual grazing-induced BVOC fluxes could be smaller than the harvest-induced fluxes, even though annual grazing events happen two times more often than the harvest events. Not being able to measure during the first three hours following the grazing event and failing to quantify all the detected compounds are the two largest limitations in the present research. Future research should take into consideration these drawbacks. Nevertheless, the present study provided relevant information on BVOC exchange between a managed grassland and the atmosphere. This kind of investigations has to be performed in different parts of the world to find out how vegetation composition and environmental conditions affect the grazing-induced fluxes and to clearly understand the controlling parameters of fluxes. On top of that, since grazing induces high emissions and changes BVOC compositions, this information has to be incorporated in the BVOC emission models to be further used in regional and global chemistry and climate models for assessing the effect of BVOC fluxes on present and future air quality and climate.

# S5 Supplementary information

## S5.1 BVOC fluxes and environmental parameters

BVOC fluxes as a function of different environmental parameters and fluxes of $CO_2$ and $H_2O$ are shown in Fig. S5.1.

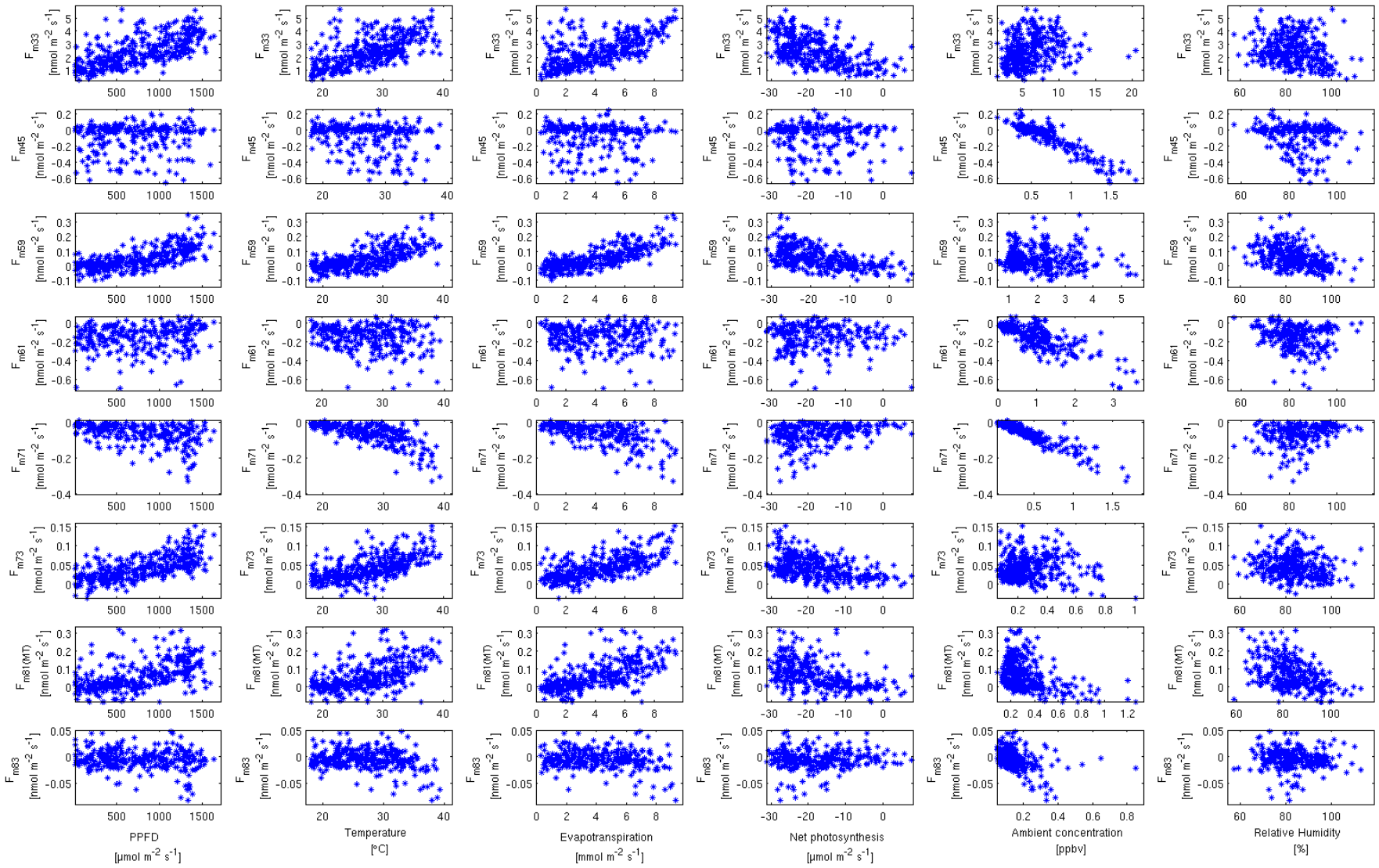


Figure S5.1 : BVOC fluxes as a function of different environmental parameters and fluxes of $CO_2$ and $H_2O$.

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# Chapter 6: Overall conclusions and perspectives

Huge amounts of biogenic volatile organic compounds (BVOCs) are exchanged between terrestrial vegetation and the atmosphere, and they are known to influence atmospheric chemistry and the climate system by participating in the atmospheric gas phase oxidation chemistry. This leads to the formation of air pollutants and/or radiatively active compounds like tropospheric ozone, secondary organic aerosols and cloud condensation nuclei, and impacts methane's lifetime by affecting the atmospheric oxidants concentration. In order to estimate the impact of BVOC exchanges on the present and future atmospheric composition and radiation budget, the study of the response of BVOC fluxes from plants and ecosystems to varying environmental conditions is required. This research project was set out to improve our understanding of BVOC exchanges between agricultural plants/ecosystems and the atmosphere since there is a strong lack of flux data from agricultural ecosystems in the literature and especially long term investigations are very rare. Therefore, this study was performed on maize (*Zea mays* L.), one of the highly cultivated plant species worldwide, during all leaf developmental stages and on grassland, one of the most abundant ecosystems on the global scale, with a specific focus on understanding grazing-induced fluxes. In the following sections, a brief discussion about the objectives, findings, limitations of the study and future research directions will be provided.

## 6. 1 Investigations on maize

The micrometeorological eddy covariance (EC) flux method is generally accepted as the best way to measure realistic BVOC fluxes at ecosystem scale in natural environmental conditions. However, retrieving information on the response of BVOC fluxes to variations in individual environmental parameters from those ecosystem scale measurements is very difficult due to strong covariation of environmental parameters in the field. Moreover those fluxes include BVOC exchanges between the atmosphere and different plant and ecosystems parts (leaves, roots, stems, flowers, fruit, weeds, soil, …) which further complicates the study of BVOC exchange mechanisms. To uncouple the effects of the different parameters, flux measurements from maize in the present study were performed on maize leaves in an environmental chamber under controlled environmental conditions. The BVOC exchanges were measured using dynamic flow-through enclosures and an on-line high sensitivity quadrupole-based proton transfer reaction mass spectrometry (hs-quad-PTR-MS) instrument for maize leaves at four different leaf developmental stages: young, semi-mature, mature, and

senescent. The measurements reported in this study mainly focussed on the light dependency of BVOC emissions at different leaf developmental stages. Temperature in the environmental chamber was kept constant at 25 °C and the plants were mostly subjected to the same symmetric stepwise varying photosynthetic photon flux density (PPFD) pattern. Depending on the PPFD conditions, temperature in the dynamic enclosures varied between 23-27 °C. Fluxes were always expressed as an amount of compounds (either expressed in molar or mass units) crossing a unit leaf surface area per unit of time.

Our first objective was to find out ***how BVOC flux intensity, pattern and spectra varied at the different leaf developmental stages of maize***. It was found that young, semi-mature and mature maize leaves all exchanged methanol, acetaldehyde, acetone, butanone/butanal, and monoterpenes with the atmosphere. In addition to these compounds (except monoterpenes), hexenals, hexenols, acetic acid and C5 alcohol/aldehyde (pentenol/pentanal) fluxes were also observed for senescent maize leaves. The above mentioned compounds are not the comprehensive list of BVOCs that can be exchanged for the maize leaves, they are the quantified exchanged compounds. The drawback related to not being able to quantify all the exchanged compounds is discussed in detail in section 6.3. Still, from the observations it can be said that at different times in the growing season, the same maize field is clearly a source of different sets of BVOCs. This ontogenetic dependence on the composition of BVOC emissions from maize should be considered when improving BVOC emissions algorithms.

BVOC flux intensities were also noticed to vary among the different leaf developmental stages. For instance, methanol fluxes were noticed to reduce with leaf maturity: daily fluxes for young leaves were around 17 times higher than those of semi-mature and mature leaves. These observations are in line with studies on other plant species (e.g. MacDonald and Fall, 1993 and Hüve et al., 2007). In addition to these observations, the maximum daily methanol fluxes for senescent leaves were around half the daily fluxes for young leaves but still were around 9 times higher than the daily fluxes for semi-mature and mature leaves. The maximum daily BVOC fluxes for senescent leaves were considered for these comparisons since the flux intensities varied strongly during the chlorosis period. The maximum daily fluxes of acetone for senescent leaves and the daily fluxes for young leaves were of the similar magnitude but were around 3.6 and 1.6 times higher than the daily fluxes for semi-mature and mature leaves. Similar as for acetone, the maximum daily fluxes of butanone/butanal for senescent leaves and the daily fluxes for young leaves were of similar magnitude but were around 2.5 and 3 times higher than the daily fluxes for semi-mature and

mature leaves. Although higher acetone emissions for young leaves have already been observed for other plant species (Aalto et al., 2014; Bracho-Nunez et al., 2011), higher butanone/butanal emissions for young leaves have not been reported yet. Monoterpenes fluxes were noticed to decrease with the developmental age of the leaf, and daily emissions from the young leaves were about 9 and 15 times higher than those from the semi-mature and mature ones, respectively. BVOC flux intensity not only varied among the different leaf developmental stages but also within a stage. For instance, a gradual increase of BVOC fluxes with leaf chlorosis was observed for the senescent leaves. Therefore, at different times in the growing season, the same maize field could have a different flux intensity for the same compound at similar environmental conditions. In MEGAN2.1 (Guenther et al., 2012), empirical coefficients describing the relative BVOC emission rates for new, growing, mature, and senescing leaves are already incorporated for the estimation of the leaf age emission activity factor. Those emission rates are compared to the one from mature leaves and therefore the empirical coefficient for mature leaves is by definition 1. For methanol for instance these coefficients are 3.5, 3, 1 and 1.2 for new, growing, mature, and senescing leaves, respectively. However, no precise information is provided in Guenther et al. (2012) about the characterization of those stages in terms of leaf age. A comparison between the empirical leaf age coefficients for the different compounds in MEGAN2.1 and the relative emission rates for the different leaf developmental stages obtained in the current investigations is therefore not straightforward and will require further investigations, mainly on BVOC emissions from freshly appearing leaves. Such investigations, however, are technically very difficult to realize for maize leaves. If we consider the young and mature maize leaves in our investigations as the "growing" and "mature" leaves in MEGAN2.1, then the corresponding empirical coefficients for maize should rather be 17 and 1 than 3 and 1 for "growing" and "mature" maize leaves, respectively. As BVOC emission rates were found to vary considerably during the senescent stage, providing an empirical factor for senescing maize leaves would not be straightforward either. Additional investigations are definitely required for a better parameterization of this leaf age factor which may be plant species dependent.

Apart from the differences in BVOC flux intensities among the different developmental stages of maize leaves in the present study, strong differences in BVOC intensities were also observed among the scarcely available studies that were performed on this crop species. Overall the observed BVOC fluxes for mature leaves in our study were rather small

compared to those from the other studies except for the study of Bachy et al. (2016) on the same maize variety for which a better agreement was found and for a very recent study by Wiß et al. (2017) who found similar menthanol emission rates from (mainly) mature leaves as in the current study. The variation in flux intensity among the studies is not well understood yet but might be related to differences in environmental conditions, and management practices and maize varieties. Indeed, differences in emission rates due to intra-genotype variability have been already mentioned in the literature (Persson et al., 2016). Unlike the mature leaves, the total accumulated BVOC emissions for senescent maize leaves were noticed to be among the highest for drying/senescent grass species mentioned in the literature. This finding needs to be verified under field conditions with other naturally senescing grass species.

In respect of diurnal BVOC flux pattern, methanol, acetone and butanone/butanal showed a similar flux pattern for the semi-mature and mature developmental stages, whereas different flux patterns were observed for the young and senescent (during chlorosis) leaves. However, monoterpenes showed a similar flux pattern for the young, semi-mature and mature leaf developmental stages. These variations in BVOC flux pattern among the developmental stages under the same environmental conditions implies that the factors controlling the production and flux were also varying with the developmental stages.

The second objective of this study was to find out ***the environmental and physiological controlling parameters driving the methanol fluxes***. Diurnal methanol fluxes for the young maize leaves were found to be complex and didn't show a clear influence of light and temperature conditions. They showed a steadily increasing trend during most of the day, indicating that the flux was controlled by some other factor than light and temperature, such as a diurnal variation in leaf growth rate or Pectin Methyl Esterase (PME) activity. Transient peaks of methanol emission were noticed at every stepwise increases in PPFD, which was also noticed by other investigators. These transient emission peaks are related to a transfer of methanol from the non-specific liquid storage pool in the leaves to the gaseous pool, following a sudden increase in stomatal conductance. Moreover, transient emissions of acetone and butanone/butanal following dark/light transition were also noticed for the semi-mature and mature stages. This suggests that stomatal conductance also has a controlling influence on these water soluble compounds fluxes. Beside daytime transient peaks of methanol emissions, an emission burst following light/dark transition was observed, for which guttation was put forward as a potential source. Although guttation is a commonly

observed phenomenon for young maize and other agricultural crop species in the evening after stomatal closure, no one has ever mentioned that methanol could come out with this water flow from xylem. More investigations are definitely needed to unravel the controlling factors of methanol fluxes from young leaves. Combined measurements of methanol fluxes and leaf growth might help to improve the understanding of methanol fluxes for young maize leaves. Without this information, methanol fluxes from young leaves cannot be estimated using an existing model. Methanol fluxes for semi-mature and mature leaves, on the other hand, seemed less complex. No nighttime emissions were observed, the fluxes followed pretty well the stepwise changes in PPFD and transient emission peaks were observed following stepwise increases in PPFD. Although methanol production in leaves was suggested to depend only on temperature (Harley et al., 2007), a production function depending both on light and temperature had to be invoked in order to obtain a good agreement between our observations and estimated fluxes with the dynamic model of Niinemets and Reichstein (2003a, b). Similar studies on this and other crop species are required for validation. For the senescent leaves, the co-occurrence between methanol fluxes and chlorosis indicates that cell breakdown is also an important physiological process which influences methanol flux. However, during the chlorosis period neither light nor temperature conditions showed a good correlation with methanol fluxes. Therefore, methanol fluxes from senescent leaves (during chlorosis) cannot be estimated using existing algorithms. Further investigations are necessary to understand its production and flux mechanisms to develop a new algorithm specifically for senescent leaves. Since methanol fluxes increased with the decrease of net photosynthesis rates for a senescent leaf, an algorithm incorporating the chlorophyll content of the leaf (senescence marker) might be useful to estimate methanol fluxes.

The third objective of this study was to ***estimate the contributions of the different developmental stages to the total methanol emission by a maize leaf/plant***. The total amount of methanol emitted by different developmental stages of a maize leaf was estimated for the first time and it was 11.4±0.9 (60±3%), 3.1±0.9 (17±5%) and 4.4±1.2 (23±6%) µmol by the young-growing, mature, and senescent $7^{th}$ leaf, respectively. In this estimation, a $7^{th}$ leaf from the base of the stem was chosen because it was one of the longest leaves of the plant and consequently, it was possible to enclose a large biomass. The total amounts of methanol emitted by all the leaves of a plant at those leaf developmental stages were also estimated and were 150±12 (64±3%), 30±21 (13±9%) and 56±17 (24±6%) µmol, respectively. This

information could be useful to the modellers for upscaling total methanol fluxes from maize leaves at field scale. However, care must be taken since these observations are specific to the variety and environmental conditions used in the investigations. Moreover, methanol fluxes from fruits, flowers and soil are also not included in this estimation.

## 6. 2 Investigations on grassland

The investigations were performed on a managed grassland in Belgium. Two side-by-side patches of grassland with equal vegetative composition were used for these investigations, one being grazed and the remaining one being undisturbed. Grazing-induced and constitutive BVOC fluxes were measured in parallel for these patches using automated dynamic flow-through enclosures and the hs-quad-PTR-MS instrument. The flux measurements were followed for these patches for ca. 10-15 days after the grazing event.

The first objective of the investigation was to find out the ***most dominant BVOCs from the undisturbed grassland and their flux intensities and controlling parameters***. The quantified most dominant BVOCs for the undisturbed grassland were methanol, acetaldehyde, acetone, acetic acid, methyl vinyl ketone/methacrolein, butanone/butanal, hexenals/monoterpenes and hexenols/hexenyl acetates. The flux intensities and directions were markedly different for these compounds. For instance, methanol and butanone/butanal were emitted during daytime, acetone and monoterpenes were deposited from late afternoon to early morning and emitted during the rest of the day, and acetaldehyde, acetic acid, methyl vinyl ketone/methacrolein and hexenols/hexenyl acetates were deposited all the time. The BVOC with the highest exchange rates was methanol followed by acetic acid and acetaldehyde whereas hexenols/hexenyl acetates were the least exchanged compounds. Overall the observed maximum flux values were 10-50% of other investigations on grassland . Not only the flux intensities were different but also the flux directions were found to be dissimilar. Several factors may contribute to this large difference, such as strong differences in grassland species compositions, environmental conditions, soil type and soil microorganism composition.

As the ambient conditions co-vary in field conditions, finding out the controlling parameters of BVOC fluxes for grassland was difficult. The fluxes of methanol, acetone, butanone/butanal and monoterpenes showed a high positive correlation with light intensity, temperature and evapotranspiration rates. Among these parameters, temperature could directly affect their production rates and enhance partitioning of these compounds in the gas

phase in the biomass/leaf interior. Light conditions control stomatal conductance and consequently, could control fluxes of these oxygenated water soluble compounds. In addition, transpiration rates and stomatal conductance are positively linked. This is why evapotranspiration also showed a high positive correlation with the fluxes. Therefore, temperature, light conditions and stomatal conductance could be the main controlling parameters for the fluxes of these compounds. Fluxes of acetaldehyde, acetic acid and methyl vinyl ketone/methacrolein, for which depositions were observed, showed a high negative correlation with ambient concentration. Therefore, factors influencing ambient concentration like surrounding vegetation, farming activities (e.g. production and use of silage, fertilization) and photochemical production of compounds indirectly affect the exchanges of these compounds for an undisturbed grassland. As a consequence, even if two grasslands are composed of the same species, compound flux intensities and directions could be different. Overall the drivers of BVOC fluxes from the undisturbed grassland were not clearly understood. Some recommendations for future research regarding this matter will be provided in the section on prospectives.

The second objective of the study was to find out ***how grazing-induced BVOC fluxes differ from the constitutive fluxes both qualitatively and quantitatively***. While daytime depositions of acetaldehyde, acetic acid and methyl vinyl ketone/methacrolein/pentanol were noticed for the undisturbed grassland, emissions were observed for the grazed grassland. Upon herbivory damage, the production rates of these compounds could have increased in grass species and have resulted in emissions. Conversely, production rates of these compounds in undisturbed grass species may not have been sufficiently high for their concentration in the sub-stomatal cavity to be higher than the ambient concentration. In this condition those compounds were taken up by the leaves. Although methanol, acetone and butanone/butanal were found to be emitted from both the undisturbed and grazed grassland during daytime (only during midday for acetone for the undisturbed grassland), emission intensities were higher for the grazed grassland. Production of these compounds must have strongly increased in the grazed grassland since a much smaller amount of biomass in the grazed grassland (about one third of that in the undisturbed grassland) gave higher emission values than the undisturbed grassland. Emissions of hexenals and hexenols, typical stress-induced compounds, were observed for the grazed grassland, while their emission rates were negligible for the undisturbed grassland. Therefore, grazing could alter BVOC flux spectra and strength of a grassland. Since more than half of the earth's agricultural land is grazing

land, grazing could have a large effect on the global BVOC budget. Thus, this kind of investigation demands more attention from the atmospheric scientist community.

The third objective of the investigation was to find out ***how BVOC fluxes vary between two grassland management practices, i.e. rotational grazing in the present study and seasonal harvest (for hay production) in the available literature***. As there is no information on grazing-induced BVOC fluxes in the literature, grassland harvest (hay production) experiments (Ruuskanen et al., 2011; Davison et al., 2008; Brunner et al., 2007; Bamberger et al., 2010) are the closest investigations against which the observations obtained in the current study can be compared. We noticed that grazing-induced fluxes were typically one to two orders of magnitude smaller than the harvest-induced fluxes. However, as flux measurements were started about 3 hours after the end of the grazing event, the flux values for the grazed grassland should be considered as lower limits. As considerably higher amounts of BVOCs are emitted after a grassland harvesting event than after a grazing event and because the number of grazing events in a rotationally grazed grassland (6 times a year for the DTO site, personal communication Louis Gourlez De La Motte ) is only twice as high as the number of harvesting events (3 to 4 times a year for a grassland site in Austria according to Bamberger et al., 2010), annual grazing-induced BVOC fluxes are expected to be significantly smaller than harvest-induced fluxes.

## 6.3 Limitations of the study and future perspectives

Although this study provides the very first investigation on BVOC fluxes from maize at all leaf developmental stages which has brought insights on intensity, pattern and spectra of BVOC fluxes from this important crop species, and also provides the very first investigation on grazing-induced BVOC fluxes, it has some limitations as well. Based on the current observations and its shortcomings, and available information in the literature, some perspectives for future research will be presented.

### 6.3.1 Limitations

#### 6.3.1.1 Limitations related to the choice of analytical instrumentation

The analytical instrument which was used to measure BVOC concentrations both in the environmental chamber and in the grassland study was a quadrupole-based PTR-MS instrument. During the last two decades the application of the PTR-MS technique has resulted in huge advancements in the field of VOC research (Ellis and Mayhew, 2014). It is a fast and sensitive online measurement technique which requires no sample accumulation and

produces almost no sampling artifacts. However, it lacks unambiguous identification of BVOC compounds. When $H_3O^+$ ions are used as the primary reactant ions in this instrument, protonated isobaric/isomeric compounds have the same nominal mass and therefore, cannot be distinguished. Moreover, protonated molecules can have the same nominal mass as dissociative proton transfer product ions originating from other molecules with a higher molecular mass. A potential way to distinguish between isobaric compounds could be by using other reactant ion species in the SRI-PTR-MS configuration (e.g. MVK/MACR using $NO^+$ ions) but this is rather the exception than the rule. $NO^+$ has not been used as primary reactant ion species in the present study. A higher degree of identification can be obtained by using in parallel chromatographic techniques (e.g. GC/MS or HPLC/MS) which have excellent compound separation capabilities but which are time-consuming, prone to sampling artefacts and have a lower temporal resolution. In the present study parallel GC-MS was performed on several occasions, but this was mostly unsuccessful as it was only able to detect C5 and higher number of carbon compounds. Tenax TA carbon mesh was used as an adsorbent in the adsorption tube but most of the exchanged compounds in these investigations were C1 to C4, except for the green leaf volatiles. Consequently, we had to rely upon literature data for compound identification.

#### 6.3.1.2 Limitations related to the investigations on maize in the environmental chamber

As a result of the fast growth rate of the young maize leaves and the stem, it was not possible to fully or partially enclose individual young leaves because of potential damage during and after enclosure. Because of the height of the mature maize plants compared to the dimensions of the growth chamber, it was not possible to enclose whole maize plants either. Consequently a compromise had to be found and it was decided to enclose full plants to study emissions from young leaves and to limit the investigations on semi-mature up to senescent leaves on the $7^{th}$ leaf of the maize plants (numbering starting from the base of the plant) for ease of enclosure without leaf damage.

A further limitation of the present study was that only BVOC fluxes were measured from maize leaves whereas a recent study on maize by Wiß et al. (2017) pointed out that fruits and flowers are also a potential source of BVOCs from this plant species.

For practical reasons the focus was put on studying the light dependency of BVOC fluxes at constant temperature conditions in the environmental chamber. It would have been interesting to perform measurements at different temperatures as well, but in order to deduce

the impact of leaf age on the emissions all plants were grown in the environmental chamber and kept at the same conditions (same T and same diurnal PPFD pattern). To investigate fluxes for young to senescent leaves, the temperature conditions in the growth chamber had to be kept constant for about three consecutive months. To get enough replicates the same investigation was performed on several occasions and finally there was no time left to investigate temperature dependency of BVOC fluxes.

During the investigations BVOC free air was supplied to the plants in order to keep background concentrations to a minimum in order to be able to quantify small fluxes. In this way our study was limited to emissions from maize plants, but in natural environmental conditions many of the compounds emitted by maize leaves can also be taken up by the leaves and the net fluxes strongly depend on the concentration of the compound in the ambient air.

An artificial light source was used in the environmental chamber. Relative to the solar spectrum, light at the ultraviolet and infrared region was totally absent for the LED lamps used in the environmental chamber. The absence of ultraviolet light might have been beneficial for the plants as it could have damaged the plant cells (Hollósy, 2002) and increased BVOC emissions from the plants (Tiiva et al., 2007). On the other hand, the effect of infrared light on plants and BVOC emissions is not yet elucidated in the literature.

Compared to the field conditions, plants grew relatively faster in the environmental chamber. Stamen appeared around 15 days earlier for the plants grown in the environmental chamber than those grown in the field (Bachy et al., 2016). One of the reasons for the faster growth of plants in the environmental chamber may be related to the high temperature conditions both during day and night compared to the field (lack of thermoperiodism). Moreover, there was no competition among the plants for light, soil water and nutrients. This faster growth rate of plants may have positively affected the methanol emission rates.

Despite the limited variation in environmental parameters and the often complex diurnal BVOC flux patterns, we were still able to provide interesting information on the influence of PPFD variations on BVOC emissions, especially for methanol. However, a better understanding of the strongly asymmetric diurnal methanol flux pattern from young shoots and the complicated temporal evolution of the BVOC fluxes from senescent leaves might have benefited from additional plant physiological measurements (e.g. very accurate and high time resolution measurement leaf growth, chlorophyll content measurements, frequent microscopic analysis of senescent leaf cell degradation), which were either not available or

incompatible with continuous enclosure of the leaves during the different measurement periods.

#### 6.3.1.3 Limitations related to the field experiments at the DTO site

Despite the fact that the first grazing-induced BVOC exchange measurements can be considered as a success, a few limitations were encountered during those experiments as well. First of all, it took about three hours to set-up the dynamic enclosures and their corresponding set-up after the grazing event. After this period the initial bursts of BVOC emissions were already strongly dampened and consequently the accumulated induced emissions of those compounds were underestimated. In addition, the driving parameters for the grassland BVOC fluxes were also not clearly understood, since the environmental parameters were strongly co-varying in the field. Moreover, the grazing-induced BVOC exchange measurements were limited to the period from 16/08/2017 to 23/09/2017, and accumulated BVOC fluxes might vary along the seasons due to variations in environmental conditions, grassland species composition and plant developmental stages. Furthermore, during the seven minutes of air sampling from the enclosures, ambient concentrations of BVOCs, $H_2O$ and $CO_2$ were assumed to be equal to those measured during the background air measurements. This may have not been always the case for all the measured flux values and could have caused small errors in the net estimated flux values from the enclosed vegetation. Additionally, after the grazing event, the ion signal at m/z 81 was totally assigned to hexenals, and this asumption may have resulted in an underestimation of monoterpenes emissions from grazed grassland.

### 6.3.2 Perspectives

#### 6.3.2.1 Use of more advanced analytical instrument to improve BVOC identification

The problems encountered in this study could be partially overcome by using an upgrade of the PTR-quad-MS, more particularly the PTR-TOF-MS in which the quadrupole mass spectrometer has been replaced by a time-of-flight mass spectrometer. Whereas mass spectra are obtained in a sequential way in a quadrupole-based instrument, which results in a low duty cycle, all ions (reactant and product ions) are detected quasi-simultaneously in the TOF version. Moreover, ion transmission for the PTR-TOF-instrument is much higher at the high mass range than for the quadrupole-based PTR-MS. This results in a much more sensitive detection of the high mass compounds. Moreover, the biggest advantage of the PTR-TOF-MS technology is its high mass resolution which allows distinguishing isobaric product ion

species. The latest PTR-TOF-MS instruments even incorporate a fast GC instrument which greatly helps in identifying the nature of the exchanged BVOCs. Application of this kind of highly valuable but very expensive instrumentation would have been a clear asset to our studies.

#### 6.3.2.2 Future efforts should focus on increased understanding of BVOC production and exchange mechanisms

As stated in section 6.3.1.2, additional measurements of plant traits or supplementary plant physiological measurements might be contemplated in future experiments for a better apprehension of the dependencies of BVOC production and emission on environmental conditions and leaf age. It is well known that methanol production in young leaves is due to growth-related changes in cell wall structure, and future attempts to parameterize methanol emissions from young maize leaves might consider simultaneous methanol flux measurements and high temporal resolution leaf area measurements. Parameterization of BVOC fluxes from senescent leaves might also benefit from simultaneous non-destructive physiological measurements (e.g. chlorophyll fluorescence), at the risk of potential damage during temporary disclosure and enclosure of the leaf. In addition to a limited understanding of BVOC emissions from maize and grassland, the production mechanisms of many of the emitted BVOCs (e.g. acetone, butanone, butanal, acetic acid) are not clearly described in the literature yet. Without a proper knowledge of those production mechanisms, it is hard to understand the BVOC fluxes and to estimate them using state-of-the-art dynamic emission models. Therefore, future investigations definitely need to be directed towards a better understanding of BVOC production mechanisms. A way to find out whether emitted BVOCs result for instance from de novo synthesis or are released from specific storage structures could be through analysis of the isotopic composition of the emitted compounds following short-time fumigation of the enclosed biomass with $^{13}CO_2$ (Shao et al., 2001; Ghirardo et al., 2010).

In order to increase the understanding on BVOC deposition to maize shoots or grassland, experiments in controlled environmental conditions could be contemplated in which the enclosed biomass is fumigated with zero air to which the BVOCs of interest are added in controlled amounts. This has already been done for isoprene and monoterpenes on grassland (Spielmann et al., 2017), but deposition studies of oxygenated VOCs would definitely be of large interest and similar studies on maize are non-existing.

#### 6.3.2.3 Alternative approaches might lead to a better capture of BVOC fluxes following grazing

Besides using dynamic enclosures, eddy covariance BVOC flux measurements might be an alternative way to study the effect of grazing on BVOC fluxes for grassland ecosystems. As a matter of fact EC BVOC flux measurements from the grazed grassland at the DTO site have been carried out continuously during the 2014 and 2015 growth season. Data analysis is still ongoing but first results do not allow a clear identification of grazing-induced fluxes, observed fluxes being very well explained by abiotic variables like light and water fluxes. However, this method is probably only successful if a large number of cows can be confined for a short time to the smallest possible footprint area to assure quick and homogeneous grazing. Without this confinement, the measured grazing-induced fluxes could be smeared out over time and might be too small for proper quantification.

#### 6.3.2.4 Representativity of the current observations on maize and grassland

The experiments carried out in the environmental chamber were performed under specific environmental conditions and one may wonder whether the obtained results are relevant for field conditions. In order to find out the representativity of the environmental chamber experiments it would be interesting to perform enclosure-based BVOC flux measurements from maize at all leaf developmental stages under field conditions as well. Especially the emission of methanol from maize through hydathodes (by forming guttation) needs to be tested under field conditions. As other important crop species (e.g. wheat) also produce guttation at dusk and dawn, similar tests could be performed on those as well. Moreover, numerous maize varieties are cultivated worldwide under different environmental conditions. Given the lack of understanding of the huge variations in BVOC fluxes from maize among the scarcely available studies, investigation of fluxes from different maize varieties at the same environmental conditions could be very interesting. Moreover ecosystem scale BVOC flux measurements from highly cultivated maize varieties in regions where they are cultivated massively can be very useful to find out the global importance of BVOC exchanges from this crop species. Not only maize but also other highly cultivated crop species need to be investigated in a similar way to constrain BVOC fluxes from these plant species. Moreover, depending on weather conditions, species composition in grasslands can be different in different parts on earth. Due to differences in grass species composition and environmental conditions, BVOC spectra and intensity could differ among these ecosystems. Therefore,

grazing-induced BVOC studies would best be performed in different regions of the world where grazed grassland ecosystems prevail.

#### 6.3.2.5 Future investigations over crop fields or grasslands should include all potential BVOC sources and sinks

Besides constitutive emissions from plant shoots or depositions to vegetation, BVOC exchanges between the atmosphere and crop or grassland ecosystems can have several sources and sinks. Plants may for instance suffer from different kinds of biotic (herbivory, microbial infestation) and abiotic (e.g. drought, heat, ozone) stress, which may give rise to stress-induced BVOC emissions (Penuelas and Staudt, 2010; Jardin et al., 2015). As limited studies are available on these matters in the literature (Vaughan et al., 2017), future investigations are definitely required. In the current study, we only followed one type of biotic stress on grassland (grazing), but future studies could be directed to the other abovementioned stresses both on crops and grassland.

As soil is an integral part of a maize field and grassland, BVOC exchanges between the atmosphere and the soil should not be neglected either when considering all potential BVOC sources and sinks in those ecosystems. Simultaneous BVOC flux measurements from enclosed maize shoots and shoots with soil could be performed to study the influence of soil on the total flux. Similar investigations can also be designed on a grassland. In addition, studies could be carried out on the use of fertilizers on BVOC fluxes from grassland. Fertilizers are known to increase BVOC emissions (Hörtnagl et al., 2011) and the application of slurry on a managed grassland, which is a common practice here in Belgium, could bring additional biodiversity to the microorganism community in the grassland and therefore influence the BVOC fluxes (Hörtnagl et al., 2011). Moreover, microorganisms living around maize and grass roots (Peiffer et al., 2013) could influence leaf scale BVOC fluxes (D'Alessandro et al., 2013) and effects of these root microorganisms on BVOC fluxes have to be investigated as well.

Finally, some important practices during maize cultivation could be significant BVOC sources. For example, during the harvest of maize at the end of the growing season, a huge amount of BVOCs is expected to be released into the atmosphere by cutting the plants. To find out the potential importance of harvest-induced BVOC fluxes from a maize field, eddy covariance ecosystem scale measurement during and after the harvest could be contemplated. Processing of maize during the harvest is thought to be a huge temporary source of BVOCs and preliminary flux measurements from maize silage were already performed in this project

(data are not presented in this thesis) during a short dedicated experiment at the DTO site using existing dynamic flow-through enclosures of which the bottom was separated from the grassland by a PFA foil and where silage material was introduced right after harvest. First results showed large emissions from silage for a large number of BVOCs, and therefore, deserve further investigations. Furthermore, storage and handling of silage for animal feeding can also lead to additional BVOC production (Hafner et al., 2013).

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**2017** Mozaffar, A., Schoon, N., Digrado, A., Bachy, A., Delaplace, P., du Jardin, P., Fauconnier, M., Aubinet, M., Heinesch, B. and Amelynck, C. : Grazing-induced BVOC fluxes from a managed grassland. European Geosciences Union (EGU), Vienna, Austria, 2017.

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## Seminar presentations

**2016** Mozaffar, A. : Methanol emissions from maize: Ontogenetic dependence to varying light conditions and guttation as an additional factor constraining the flux, inter-faculty seminar in Gembloux, 2016.