Biogenic volatile organic compound emissions from senescent maize leaves and a comparison with other leaf developmental stages

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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 of 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 healthy green leaves. Senescent leaves of grain crop species could 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 most 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 (31% of the total cumulative BVOC emission on a mass of compound basis) and acetic acid (30%), followed by acetaldehyde (11%), hexenals (9%) and m/z 59 compounds (acetone/propanal) (7%). 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 senescence-related BVOC sources. Total cumulative BVOC emissions from senescent maize leaves were found to be among the highest for senescent Poaceae plant species. BVOC emission rates varied strongly among the different leaf developmental stages, and senescent leaves showed a larger diversity of emitted compounds than leaves at earlier stages. Methanol was the compound with the highest emissions for all the leaf developmental stages and the contribution from the young-growing, mature, and senescent stages to the total methanol emission by a typical maize leaf was 61, 13, and 26%, respectively. This study shows that BVOC emissions from senescent maize leaves cannot be neglected and further investigations in field conditions are recommended to further constrain the BVOC emissions from this important C4 crop species.

1 Introduction

Terrestrial vegetation is a huge source of volatile organic compounds (VOCs) in the Earth's atmosphere. Besides playing a role in plant biology and ecology (Pierik et al., 2014), biogenic VOCs (BVOCs) are generally highly reactive with the major atmospheric oxidants, thus affecting the oxidation capacity of the atmosphere, air quality and climate (Atkinson, 2000; Laothawornkitkul et al., 2009; Pacifico et al., 2009). BVOC emissions from plants are highly species-specific and not only depend on environmental conditions (Guenther et al., 2012) and abiotic or biotic stress factors (Holopainen et al., 2010) but also on plant ontogeny (Bracho-Nunez et al., 2011). Therefore they should be investigated at all developmental stages, including senescence.

Leaf senescence is the final stage of leaf development. It is a complex energy-dependent self-digesting process that facilitates the remobilisation of nutrients from the senescing leaf to growing vegetative plant organs or developing seeds and fruits, where they are reused for biosynthesis (Woo et al., 2013; Keskitalo, 2005; Gan and Amasino, 1997; Taiz et al., 2015). Under normal growing conditions leaf senescence is governed by the developmental age of the leaves, which is a function of hormones and other regulatory factors (Taiz et al., 2015). Under unfavourable environmental conditions (e.g. drought or enhanced ozone concentrations) or biotic stress (e.g. pathogen infestation), however, the leaf senescence process can occur prematurely. Three main phases are generally distinguished in the developmental leaf senescence process (Taiz et al., 2015). The initiation phase is characterised by a gradual decline in photosynthesis and a transition of the leaf from being a nitrogen sink to a nitrogen source. Self-digestion of cellular constituents and macromolecules mainly occurs during the second phase, the degenerative phase. The third phase, the terminal phase, is characterised by loss of cellular integrity, cell death, and finally (in most cases) leaf abscission.

Although numerous studies have already been performed on 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 Gas Chromatography – Mass Spectrometry (GC-MS) sample every 3 days) to adequately represent the emission dynamics. Therefore, additional studies at increased time resolution are required for a better characterisation of BVOC exchanges between senescent leaves and the atmosphere during the whole senescence period.

To assess the importance of BVOC emission rates from the senescent leaves of a plant, information about BVOC emission rates from other leaf developmental stages (young, semi-mature, mature) is also necessary. In this study we will mainly focus on BVOC emissions from senescent maize (*Zea Mays L.*) leaves, but we will also compare them with BVOC emissions from other developmental stages of maize leaves measured under the same environmental conditions. Despite being a vastly cultivated crop species worldwide, only a few literature studies have been devoted to BVOC emissions for this 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, data on BVOC emission rates from senescent maize leaves are missing in the abovementioned literature.

Maize is a monocarpic (a plant which only flowers and bears fruit once in its lifetime) herbaceous C4 plant for which whole plant senescence occurs with seed maturation (Lim et al., 2007). However, the first leaf at 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. 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 order to improve the knowledge on BVOC emissions from this important crop species we aim to provide answers to the following specific 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 species of the Poaceae family, 3) how do BVOC emission rates vary among the different leaf developmental stages of maize, and 4) what are the

contributions of the different developmental stages to the total emission of individual BVOC compounds by a maize leaf/plant.

2 Materials and methods

2.1 Plants and environmental conditions

The 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 day old maize plants (age counting began at seed germination) were used. The shoots of the young maize plants were completely enclosed because it was not feasible to enclose a single young leaf for a sufficiently long period without damaging it due to the fast elongation rate of both leaves and stem. The upper part (ca. 55 cm starting from the tip) of almost fully developed, healthy 7^{th} leaves of 30-40 day old plants (around 120 cm tall) and the fully developed (7^{th}, 8^{th} or 9^{th}) leaves of 60-70 day old, fully grown plants (around 180 cm tall) were enclosed to measure BVOC emission rates from semi-mature and mature leaves during 5 consecutive days, respectively. The above-mentioned leaf numbers refer to the order in which new leaves appear during plant development and leaf numbering thus starts from the base. The choice for (mainly) 7^{th} leaves was determined by their position with respect to the ground and the ceiling level of the environmental chamber and the possibility to enclose them without damaging them. Senescence-induced BVOC emission rates were measured from senescing (7^{th}, 8^{th} or 10^{th}) leaves of fully grown 60-95 day old plants. The upper half of those leaves was enclosed a few days before the start of the senescence initiation phase, which was identified by a decrease of photosynthesis (Taiz et al., 2015) and preceded chlorosis, i.e. leaf yellowing due to chlorophyll degradation, by a few days. Chlorosis started at the tip of the leaf and gradually progressed towards the base of the enclosed leaf within 5-10 days (range for 10 replicates). The onset and evolution of leaf chlorosis, however, could only be determined visually and not by a quantitative metric (e.g. photochemical efficiency or chlorophyll content). When describing the BVOC emission rate dynamics in Section 3.1, the term “chlorosis period” refers to the period between the first visual signs of yellowing and the total absence of green areas on the enclosed part of the leaf. Whereas

the period between the onset of the decrease of photosynthesis and the onset of chlorosis can be considered as the initiation phase of leaf senescence, the chlorosis period largely coincides with the degenerative phase. The terminal phase can be mainly associated with the post-chlorosis period (from the end of the chlorosis period to the end of the measurements). It is to be noted that the third phase of senescence in maize does not imply leaf abscission. In order to support the description of the temporal evolution of senescence and BVOC emissions in this Section and in subsections 3.1.1 and 3.1.2, daily pictures of a typical senescent leaf (taken at 10 AM every day), along with the temporal evolution of the daily net photosynthesis rate and the methanol and acetic acid daily emission rates are shown in Fig. 1.

Figure 1

Investigations on young, semi-mature, and mature leaves were performed on 5 replicates for each stage, and 10 replicates were used for investigating emissions from senescent leaves. More details about the enclosed leaves at the different leaf developmental stages and the associated plant age and developmental status can be found in Table 1.

All these experiments were conducted on potted (20 L 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 fertilised before planting with NPK fertiliser (6-5-5, Substral Nutrimax, Belgium) containing micronutrients (Cu, Fe, Mn, Mo, Zn). Daytime temperature in the environmental chamber was maintained at 25°C throughout the experimental period and during the night the temperature decreased by around 2°C due to the 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 with seven hours of darkness (Fig. 2). All the plants were watered regularly to maintain a good soil moisture content (35-40%, field capacity was 40%).

Table 1: Detailed information about the enclosed leaf/leaves at the different leaf developmental stages, the associated plant age and two useful metrics, BBCH code and AGDD (Accumulated Growing Degree-Days), for the plant developmental stages at which the experiments were carried out. The BBCH code and AGDD are explained in supplement S1.

Leaf developmental stage	Enclosed leaf/leaves	Plant age [days]	BBCH code*	AGDD [degree-days]
Young	first 2-5 leaves	4-14	10-14	122-274
Semi-mature (almost fully grown)	7th leaf	30-40	17-19	518-671
Mature (fully grown)	7th/8th/9th leaf	60-70	65-69	976-1128
Senescent	7th/8th/10^{th} leaf	60-95	65-69*	976-1510

* range of BBCH codes at which the onset of senescence was identified for the enclosed leaves.

2.2 Experimental setup

Young maize shoots were enclosed in 22 L cylindrical dynamic flow-through enclosures made of transparent PFA (perfluoroalkoxy Teflon) envelope (Norton, Saint-Gobain Performance Plastics, NJ, USA). Similar 30 L enclosures were used to partially enclose the semi-mature, mature, and senescent leaves. In addition, a similar empty enclosure was used to measure the background VOC concentrations in the air that was supplied to the enclosures. 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 litre per minute) air flow sent through each of the enclosures were controlled at around 40% and 400 ppm, respectively. A conventional high-sensitivity quadrupole-based Proton Transfer Reaction-Mass Spectrometry instrument (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 and CO_2 calibrations were performed monthly. Further details about the set-up have been presented in Mozaffar et al. (2017).

2.3 Tentative identification and quantification of emitted BVOCs

The operating principle and technical details of the PTR-MS technique have been amply described in the literature (e.g. Lindinger et al., 1998; Ellis and Mayhew, 2013). In the present work, 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 (1 Td = 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 of BVOC-related ion species for which signal intensities above the detection limit have been observed in PTR-MS spectra of maize leaf emissions. The m/z values of ions which were used for emission rate quantification, along with a tentative identification of the associated BVOCs are shown in Table 2. This identification mainly relies on Karl et al. (2005) who unambiguously identified BVOCs released from drying rice (*O. sativa*) by using the hyphenated GC/PTR-MS technique. In addition to the BVOC-related ions, isotopes of the reactant ion species H_3O^+ (at m/z 21) and its first hydrate $H_3O^+ \bullet H_2O$ (at m/z 39) were monitored.

Table 2: The m/z ratios and list of potential contributory compounds for which emission rates 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 also shown.

m/z	Compound	Mixing ratio (ppbv)	
		Bottle 1[a]	Bottle 2[a]
33	**Methanol**	1074	1013
45	**Acetaldehyde**	999	969
57	**Hexenals**		1041[b]
59	**Acetone,** propanal, 2,3-butanedione	973	502
61	Acetic acid		
69	**Isoprene**, pentenol, n-pentanal	441	483
73	**Methyl ethyl ketone** (MEK), 2-methyl-propanal, butanal	494	516
81	**Monoterpenes,**	851[c]	
	Hexenals		1041[b]
83	**Hexenols**, n-hexanal, hexenyl acetates		986[d]
99	**Hexenals**		1041[b]
137	**Monoterpenes**	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 in calibration bottle 2 which resulted in product ions at m/z 81.

[c] Alpha-pinene (452 ppbv) and sabinene (399 ppbv) were used as proxies for the monoterpene family and were the only compounds in calibration bottle 1 which resulted in product ions at m/z 81.

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

PTR-MS calibrations were performed by using two 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. When several compounds potentially contributed to the ion signal at a given nominal m/z value (e.g. methyl ethyl ketone, 2-methyl-propanal and butanal at m/z 73), it was assumed that they possessed similar calibration factors. By calibrating the PTR-MS to the compound indicated in bold in Table 2 (methyl ethyl ketone for the above-mentioned example) and applying the calibration factor to the measured ion signal at that m/z, we estimated the sum of the concentrations of those compounds.

The H_3O^+/monoterpene reaction in the drift tube reactor of the PTR-MS is known to result mainly in the protonated molecule at m/z 137 and a fragment at m/z 81 and the product ion distribution is isomer-dependent (Tani et al., 2003). However, since the rate constant for proton transfer from H_3O^+ to monoterpenes is isomer-independent, the sum of the ion signals at m/z 81 and 137, weighted for their relative transmission in the mass spectrometer, is expected to be isomer-independent. This sum was used to determine the emission rate of all monoterpene isomers emitted by the young, semi-mature, and mature leaves. During the measurements with senescent leaves the ion signals at m/z 137 were always below the detection limit, even when ion signals at m/z 81 were maximal (I_{137}/I_{81} at maximum I_{81} is 0.01 ± 0.02 with $I_{137(81)}$ being the net ion signal at m/z 137(81)). This indicates that monoterpene emission rates were not significant during this stage and that ions at m/z 81 did not result from dissociative proton transfer of monoterpenes but corresponded to dehydrated protonated hexenals. In order to obtain accurate emission rates 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 also continuously monitored, both during the enclosure measurements and calibrations, and the sum of the signals of the three product ion species, weighted for ion transmission in the mass spectrometer, was used to derive the emission rate of the sum of hexenal isomers. As hexenals and monoterpenes both result in a product ion at m/z 81, calibration of the PTR-MS for those compounds required the use of two multi-component calibration mixtures containing either a hexenal or a monoterpene, in addition to the

other compounds to be quantified (Table 2). The calibration mixture containing the monoterpenes was used for the experiments on young, mature, and semi-mature leaves, whereas the mixture containing hexenal was used for the experiments on senescent leaves.

The calibration factor for acetic acid (at m/z 61), a compound which was not present in the calibration bottles, was estimated from the factor 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. The m/z 81 and 83 compounds emitted by the senescent leaves are associated to C6 volatiles (hexenals, hexanal, hexenols, hexenyl acetates) and will be designated as green leaf volatiles (GLVs) hereafter.

2.4 Emission rate computation

Unless explicitly mentioned otherwise, BVOC emission rates E_{BVOC} from maize leaves are expressed as mass flow rates per unit of leaf dry weight [µg of the compound g_{DW}^{-1} s^{-1}]. They are calculated according to Eq. 1 in which F_{air} is the molar flow rate of the air which is supplied to the enclosure, M_{BVOC} is the molar mass of the BVOC, DW is the dry weight of the enclosed (part of the) leaf, and $x_{BVOC,PE}$ and $x_{BVOC,RE}$ are the mole fractions of the BVOC in the sampled air from the plant enclosure and the empty reference enclosure, respectively.

$$E_{BVOC} = \frac{F_{air} \times \left(x_{BVOC,PE} - x_{BVOC,RE}\right) \times M_{BVOC}}{DW} \quad (1)$$

The mole fractions of the BVOC are obtained by dividing the normalised background-corrected PTR-MS BVOC ion signals $I_{m/z}$, expressed in normalised counts per second (ncps), by the BVOC calibration coefficients C_{BVOC} [ncps $ppbv^{-1}$] (Mozaffar et al., 2017).

The dry weight of the leaves was determined at the end of the experiments after at least 48 hours of drying in an oven at 75°C until all water was evaporated and a constant weight was reached. Photosynthesis and transpiration rates were obtained in a similar way but are expressed in molar units and per unit of leaf area [mol m^{-2} s^{-1}]. Leaf area was estimated as described in Mozaffar et al. (2017).

3 Results and discussion

3.1 BVOC emissions from senescent maize leaves

Significant emissions of methanol, acetaldehyde, m/z 59 compounds, acetic acid, and hexenals were observed from senescent maize leaves and their temporal evolution is shown in Fig. 2 for a single leaf.

Figure 2

Although the plants were grown and investigated under the same environmental conditions, a considerable variability was noticed in the temporal evolution of the emissions among the different replicates as is shown in Figures S1a and S1b in Supplement S1. However, the BVOC emission dynamics from the investigated leaves show similar characteristics, as will be described in the following subsections. Small emission rates of m/z 69, m/z 73 and m/z 83 compounds were also noticed (Table 3), but they often barely exceeded the detection limit. The emergence of BVOC emissions or their increase (in case the BVOC was also emitted constitutively) took place at the start of the degeneration phase. Photosynthesis and transpiration diminished gradually as chlorosis progressed from the apex towards the base of the leaf and finally stopped 2-4 days before the end of the chlorosis period (as shown in Fig. 2 for one replicate). While some of the BVOCs were predominantly emitted during and just after the degeneration phase, others were emitted during the termination phase of senescence as well, as will be discussed in detail in the following sections.

The temporal emission dynamics of the individual BVOC species, the relative contribution of their cumulative emissions to the total emission, as well as a comparison with cumulative emissions for other senescent species of the Poaceae family will be discussed hereafter.

3.1.1 Methanol, acetaldehyde and GLVs are predominantly emitted during the degeneration phase of leaf senescence

At the beginning of the chlorosis period, the emission rates of methanol, acetaldehyde, and hexenals rose slowly as leaf discoloration moved 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 emission rates occurred at around 1-3, 0-2 and 0-2 days before the end of the chlorosis period, respectively (Fig. 2 and Fig. S1a). By then photosynthesis and transpiration from the steadily decreasing green part of the enclosed leaf were already greatly reduced. Emissions of methanol, acetaldehyde, and hexenals exceeding 20% of their maximum value lasted for 3-8, 3-7, and 2-7 days, respectively. The temporal evolution of normalised m/z 83 compound emission rates was similar to that of normalised hexenal emission rates (data not shown). 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. S1a in Supplement S1) . Figure 2 also shows small methanol and acetaldehyde emissions after the chlorosis period which persisted until the end of the measurements. These emissions showed a fixed diurnal pattern with a maximum emission rate of at most 10% of the maximum emission rate during the chlorosis period.

As can be seen in Fig. 2 and in Fig. S1a, no distinct diurnal patterns were observed for methanol, acetaldehyde, and GLV emission rates during the chlorosis period and there was no clear correlation between these emission rates and the environmental conditions (temperature, PPFD) in the dynamic enclosures. This is in agreement with previous observations of emissions of these compounds from drying leaves (Fall et al., 1999; Warneke et al., 1999; 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 emissions 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. Ozuna et al. (1985) reported that the stomata of senescent

Nicotiana glauca leaves which already showed marked chlorosis (the degree of chlorosis varied from 60 to 90% of the total leaf surface area) remained closed even in the presence of light. As maximum emissions of methanol and acetaldehyde in our experiments were measured close to the end of the chlorosis period, the main emission route for those compounds during the degeneration phase of leaf senescence was therefore 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). As changes in the biomechanical properties of the epidermis cuticle have been reported for *Sonneratia alba* leaves (Takahashi et al., 2012) during this senescence phase, diffusion of those compounds through a degraded epidermal layer might be considered as a potential emission route.

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). 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 remobilisation during the degeneration phase of senescence (Woo et al., 2013; Keskitalo, 2005), massive changes occur in the primary cell wall structure, which potentially stimulates massive methanol production. Methanol emissions from the dead, brown leaves during the termination phase could be either due to the physical membranes breakdown following dehydration or result from the presence of active PME in dead plant materials (Castaldo et al., 1997; Galbally and Kirstine, 2002). Indeed, 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 known, 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 responsible for high production of acetaldehyde during the degeneration phase.

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 oxidised 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. 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.

3.1.2 Persistent m/z 59 compounds and acetic acid emissions during the terminal phase of leaf senescence

Whereas the temporal evolution of acetic acid emissions was well-marked and no emissions were observed prior to senescence, normalized 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. 2 and Fig. S1b). Normalised acetic acid and m/z 59 compounds emissions increased somewhat slower than those of methanol, acetaldehyde and GLVs, resulting in a delay of their maximum emissions with respect to the latter three compounds by 1-2 days.

Furthermore, in strong contrast to methanol, acetaldehyde and GLVs, acetic acid and m/z 59 compounds emission rates 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 night-time m/z 59 compounds emissions (acetone) from dry plant parts and litter have previously been mentioned in the

389 literature (Schade and Goldstein, 2001; Warneke et al., 2002) and acetic acid emissions from dry
390 plant material have been reported as well (Kesselmeier et al., 1998; Warneke et al., 1999; Crespo
391 et al., 2013). The production of acetone and acetic acid during the drying process following the
392 chlorosis period might be explained by the occurrence of a non-enzymatic Maillard reaction
393 (Ikan et al., 1996). After the end of the chlorosis period a significant positive correlation (R^2 =
394 0.53, $P < 0.01$) was observed between the acetic acid emissions and the relative humidity of the
395 air supplied to the enclosures. Similarly, Warneke et al. (1999) observed a large increase in
396 partially oxidised VOC (POVOC) emissions after wetting of dried biomatter. They explained
397 this by the transfer of some of the POVOC molecules, produced by the Maillard reaction and
398 remaining attached to the surface of the solid structure of the dry leaf material, to the aqueous
399 phase through replacement by highly polar water molecules. The dissolved POVOCs were
400 subsequently transferred from the aqueous to the gas phase until a gas/liquid equilibrium,
401 determined by the Henry's law constant, was reached. Although the dry leaves were not wetted
402 in our experiments, release of acetic acid molecules from the biomass surface layer may have
403 been influenced by the presence of a relative humidity dependent microlayer of water on the
404 surface layer or by interactions with polar water vapour molecules. Those interactions may have
405 promoted the release of the polar BVOC molecules from the surface layer. A short additional
406 experiment was carried out in which an inert surface, coated with a pure acetic acid microlayer,
407 was inserted in an empty enclosure demonstrated that such a positive correlation between acetic
408 acid emissions and relative humidity was not restricted to senescent biomass. Indeed, the acetic
409 acid emission rate from the coated surface, which was of similar magnitude to that of the
410 enclosed senescent maize leaves, was found to increase by a factor of 2.7 when increasing the
411 relative humidity of the purge air from 40 to 64 %. Moreover, increased emissions of acetic acid
412 and other polar compounds with increasing relative humidity have been reported previously from
413 other materials such as wood boards (Steckel et al., 2013) and indoor furniture (Fechter et al.,
414 2006; Schaeffer et al., 1996).

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

Cumulative emissions of the quantified BVOCs for the different senescence phases are shown in Table 3, along with the maximum instantaneous emissions and the maximum daily emissions that have been observed over the entire senescence period. Additional statistical information on instantaneous and daily emissions and on cumulative emissions from the investigated senescent leaves is provided in Table S2 and in Fig. S2 in Supplement S1. The emission rates for some compounds (e.g. methanol, m/z 59 compounds, and acetic acid) were still well above the detection limit at the end of the measurement period. Consequently, the reported cumulative emission values from the onset of senescence to the end of the measurement period (31 days) for those compounds should be considered as lower limits for their total cumulative emissions during leaf senescence.

Table 3: BVOC emissions from maize leaves at different leaf developmental stages. Maximum values for the instantaneous ($E_{inst,max}$) and for the daily emission rate ($E_{day,max}$), and cumulative emissions for the initiation, degeneration, and termination phases are shown for the different BVOCs emitted during senescence. Also tabulated are the daily emission rates (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. The error on the emission rates corresponds to the standard deviation (1σ).

	Senescent leaves					Young leaves	Semi-mature leaves	Mature leaves
	$E_{inst,max}$ (ng g_{DW}^{-1} s^{-1})	E_{acc} (µg g_{DW}^{-1})			$E_{day,max}$ (µg g_{DW}^{-1} day^{-1})	E_{day} (µg g_{DW}^{-1} day^{-1})	E_{day} (µg g_{DW}^{-1} day^{-1})	E_{day} (µg g_{DW}^{-1} day^{-1})
Compounds		Initiation phase	Degeneration phase	Termination phase				
Methanol	0.9 ± 0.4	14 ± 11	150 ± 30	50 ± 20	53 ± 16	137 ± 17*	4.1 ± 0.7*	4.8 ± 1.0*
Acetaldehyde	0.38 ± 0.10	1 ± 2	44 ± 12	32 ± 19	15 ± 3	-	-	-
m/z 59 compounds	0.05 ± 0.02	5 ± 5	12 ± 7	31 ± 20	2.3 ± 1.2	2.7 ± 1.1	0.4 ± 0.2	0.9 ± 0.3
Acetic acid	0.17 ± 0.05	6 ± 7	40 ± 20	160 ± 60	12 ± 4	<DL**	<DL	<DL
m/z 69 compounds	0.08 ± 0.02	3 ± 3	13 ± 8	22 ± 16	3.4 ± 1.3	<DL	<DL	<DL
m/z 73 compounds	0.03 ± 0.02	3 ± 4	5 ± 7	11 ± 15	1.2 ± 1.3	1.7 ± 1.0	0.4 ± 0.2	0.30 ± 0.08
Hexenals	0.21 ± 0.14	6 ± 7	30 ± 20	20 ± 20	10 ± 8	-	-	-
Monoterpenes	<DL	<DL	<DL	<DL	<DL	10 ± 6	0.6 ± 0.2	0.4 ± 0.2
m/z 83 compounds	0.07 ± 0.02	1.4 ± 1.7	9 ± 6	11 ± 9	2.6 ± 1.5	-	-	-

*: Mozaffar et al. (2017); ** Detection Limit

436

437 Methanol and acetic acid were clearly the compounds with the highest emissions (expressed in
438 mass of compound per leaf dry weight), accounting both for around 30% of the total cumulative
439 emission from the onset of senescence to the end of the investigations (Fig. 3a). They were
440 followed by acetaldehyde (11%), hexenals (9%), m/z 59 compounds (7%), and m/z 69
441 compounds (6%). The m/z 73 and m/z 83 compounds also made up a small fraction of the total
442 BVOC emissions from the senescent leaves, but their individual contributions were not higher
443 than 3%.

444

445 Figure 3

446

447 Fig. 3 clearly shows that the relative contribution of the emitted compounds varied among the
448 time periods considered. Differences in relative BVOC composition reflect the differences in
449 temporal emission dynamics among BVOCs that were discussed in Section 3.1.1 and 3.1.2.
450 Indeed, as methanol, acetaldehyde, and hexenals were predominantly emitted during the
451 degeneration phase (see Table 3), it is clear that those compounds together made up a large part
452 (75%) of the BVOC emissions for that period (Fig. 3b). Because of the strong persistence of m/z
453 59 compounds and acetic acid emissions after the chlorosis period, the relative contribution of
454 those compounds increased with time after that period. During the termination phase (Fig. 3c),
455 the m/z 59 compounds and acetic acid together made up half (55%) of the BVOC emissions, and
456 acetic acid was predominantly emitted during that period (46%).

457 While some studies (Karl et al., 2001b; Karl et al., 2005; Eller et al., 2011; Crespo et al.,
458 2013) have reported cumulative BVOC emissions from biomass drying and simulated
459 drying/senescence experiments, information about cumulative BVOC emissions from senescent
460 leaves that are still attached to the plant is, to our knowledge, not available in the literature.
461 Moreover, the abovementioned studies are the only available ones which report cumulative
462 emissions in conditions which somehow approximate senescence in real natural conditions.
463 Therefore we will compare the cumulative BVOC emissions from senescent maize leaves over
464 the entire measurement period against the results from those studies (Table 4), which were all
465 performed on different species of the Poaceae family. Overall, BVOC emissions from senescent

maize leaves are among the highest for the drying/senescent plant species. The cumulative methanol emission from the senescent maize leaves is very similar to that of hay, reported by Karl et al. (2001b). By contrast, cumulative 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. Cumulative 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 of black bamboo, which is the only other species of the Poaceae family for which cumulative acetic acid emissions have been reported (Warneke et al. (1999) also mentioned acetic acid emission from grass, but didn't report cumulative emissions). Maize, hay, rice, and black bamboo all have higher cumulative GLV emissions than sorghum and switchgrass.

This comparison of cumulative BVOC emissions from the different plant species should, however, be taken with caution because of differences in the way they have been determined (from leaves still attached to the plant as in this study or from cutting of biomass, followed by drying in the field or in an oven as in the other studies), in environmental conditions during the experiments, and in the length of the accumulation period (see Table 4).

Table 4: Comparison of the cumulative BVOC emissions during senescence of maize leaves and drying of hay, *Sorghum sudanense* (sorghum), *Oryza sativa* (rice), *Panicum virgatum* (switchgrass) and *Phyllostachys nigra* (black bamboo). Also mentioned in the Table are the accumulation 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 (~31 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	($\mu g/g_{DW}$)	($\mu g/g_{DW}$)	($\mu g/g_{DW}$)	($\mu g/g_{DW}$)	($\mu g/g_{DW}$)	($\mu g/g_{DW}$)
Methanol	210 ± 30	160	2 ± 0.9	3.1 ± 0.8	9.07 ± 2.35	26.1
Acetaldehyde	80 ± 30	20-80	6.6 ± 2	8.4 ± 0.7	13.99 ± 5.44	73.2
m/z 59 compounds	50 ± 30	20-40	0.4 ± 0.3	1.7 ± 1.1	2.21 ± 0.69	13.3
Acetic acid	200 ± 80	-	-	-	-	28.6
m/z 69 compounds	40 ± 20	15[a]	0.32 ± 0.13[b]	3.2 ± 1.7[b]	1.15 ± 0.53[c]	58[c]
m/z 73 compounds	20 ± 30	11-80	-	-	-	-
Hexenals	60 ± 50	100-240	0.53 ± 0.17	100 ± 49	1.49 ± 1.42	231[d]
m/z 83 compounds	21 ± 15	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 ion intensities at m/z 57, 81, 99, 83, 101, and 103; [e] hexenols and hexanal

3.2 Contribution of leaf senescence to the total BVOC emissions from a maize leaf/plant

In order to investigate the relative importance of BVOC emission rates from senescent maize leaves, these emission rates were compared with those of young, semi-mature, and mature maize leaves measured under the same environmental conditions (Table 3). Strong variations in daily emission rates were observed among the different leaf developmental stages for those compounds that could be quantified for the three earliest stages. Methanol was clearly the compound with the highest emission rates for all stages. The highest daily emission rate during the senescence period was about 40% of the average daily emission rate for the young leaves and about a factor of 10 higher than that of semi-mature and mature leaves. The high emission rates for senescent and young leaves with respect to those of the other developmental stages are due to enhanced production of methanol by PME-catalysed demethylation of pectin during cell wall remodelling. Whereas complex diurnal methanol emission profiles were found for the young leaves (Mozaffar et al., 2017), no clear correlation between the methanol emission rate and PPFD could be observed for the senescent leaves either. Maximum daily emission rates for m/z 59 and m/z 73 compounds during senescence were of similar magnitude to the corresponding average daily emission rates for young leaves and about 3-6 times higher than the average daily emission rates for the semi-mature and mature leaves. Daily monoterpene emission rates for young maize leaves were more than ten-fold higher than for semi-mature and mature leaves and emission rates from senescent leaves were below the detection limit. Higher acetone and monoterpene emissions from young leaves have previously been reported in the literature and higher defence requirements for the young leaves (Bracho-Nunez et al, 2011) and lower metabolic activity in mature leaves resulting in low *de novo* synthesis rates (Aalto et al., 2014) have been proposed as potential reasons.

Although the data listed in Table 3 allow a comparison between the maximum daily BVOC emission rates during senescence and the average daily BVOC emission rates measured during relatively short periods for the young, semi-mature, and mature leaves, additional information is required to assess the contributions of the different leaf developmental stages to the total BVOC emissions for an individual leaf in the course of its lifetime. By taking into account the

519 experimental daily methanol emission rate data from the young and mature leaves (Mozaffar et
520 al., 2017) and the cumulative methanol emissions from senescent leaves (Table 3), and by using
521 information about the relative leaf area growth rate and the duration of the young-growing
522 (encompassing young and semi-mature) and mature leaf developmental stages, it was possible to
523 estimate the cumulative methanol emissions from a 7^{th} leaf of a maize plant during the young-
524 growing, mature and senescent leaf developmental stages. The leaf number again refers to the
525 order of appearance of the leaf on the maize plant. They were 360 ± 30 µg, 80 ± 30 µg and 150 ±
526 40 µg, respectively, and the resulting total amount of methanol emitted by a 7^{th} leaf in the course
527 of its lifetime was therefore 590 ± 60 µg. The young-growing, mature and senescent stages
528 consequently contributed 61 ± 3, 13 ± 5 and 26 ± 6% to the total methanol emission from a 7^{th}
529 leaf. Details about the estimation procedure are provided in Supplement S1.

530 Based on the emission rates obtained for a 7^{th} leaf, the total amount of methanol emitted by all
531 leaves of a representative maize plant, grown in the environmental chamber at 25 °C and exposed
532 to the diurnal light pattern described in Section 2.1, has been estimated for the different leaf
533 developmental stages. Values of 4.8 ± 0.4, 0.8 ± 0.5 and 1.9 ± 0.6 mg were obtained for the
534 cumulative methanol emission from all young-growing, mature, and senescent leaves over the
535 entire lifetime of the plant, respectively. The total amount of methanol emitted by the leaves of a
536 whole maize plant from shoot emergence to full senescence was therefore equal to 7.5 ± 0.9 mg
537 and the contributions of the different stages to the total methanol emission were 64 ± 3, 10 ± 8
538 and 26 ± 7%, respectively. The estimation of the total cumulative methanol emission from a
539 whole maize plant over the course of its lifetime should however be considered with caution. As
540 already mentioned it was assumed that all leaves were exposed to the same PPFD and the effect
541 of shading by leaves with a higher leaf number was not taken into account. Moreover, a large
542 variability in the duration of the mature stage was noticed among leaves with different leaf
543 numbers, which may be related to the demands of the plant for nutrient relocation. The total leaf
544 emissions of methanol from a maize plant presented in this work could be used as a first estimate
545 for upscaling to field scale in regions characterised by environmental conditions close to those of
546 the growth chamber. However, a general upscaling from the leaf/plant level to ecosystem level
547 would definitely benefit from methanol emission measurements at different temperatures at all
548 leaf developmental stages. Moreover, uptake of atmospheric methanol by the ecosystem may

549 also be important in the field (Laffineur et al., 2012; Wohlfahrt et al., 2015) and should be taken
550 into account.

551 For the other BVOCs emitted by maize leaves, assumptions about the temporal evolution of
552 the emission rates at the different leaf developmental stages are less straightforward than for
553 methanol. Indeed, whereas methanol emission from young leaves has been associated with leaf
554 growth (Hüve et al. 2007), this is not the case for other BVOCs and therefore a simple
555 relationship between the daily emission rate of those compounds and the daily relative leaf area
556 growth rate (eq. S3 in Supplement S1) cannot be put forward. Consequently, an estimation of the
557 relative contribution of the different leaf developmental stages to the total emission from a 7th
558 maize leaf for other BVOCs than methanol- from the emission rate data obtained in this work
559 would have been prone to very large errors and has therefore not been accomplished. The same
560 applies to extrapolation to an entire maize plant.

561 # 4 Conclusions

562 Maize is one of the most cultivated crop species worldwide, but only 5 studies on BVOC
563 exchanges from maize are available in the literature and none of them deal with emissions from
564 senescent leaves. In contrast to most studies on BVOC emissions from artificially senescing
565 leaves (cutting and drying), the senescent maize leaves in our experiments were still attached to
566 the stems.

567 The main emitted compounds, ranked according to their cumulative emissions over the
568 senescence period, were found to be methanol, acetic acid, acetaldehyde, hexenals, m/z 59
569 compounds, m/z 69 compounds, m/z 73 compounds, and m/z 83 compounds. Important
570 differences were observed in the temporal emission profiles of these compounds. Whereas
571 methanol, acetaldehyde, and GLVs (hexenals and m/z 83 compounds) were emitted mainly
572 during the degeneration phase of leaf senescence, m/z 59 compounds and acetic acid emission
573 rates increased at the end of that phase and their emissions remained high during the termination
574 phase, even when the leaves were already completely dry. Beside m/z 59 compounds and acetic
575 acid, the rest of the abovementioned compounds were also emitted in small but significant
576 amounts from dry leaves during the termination phase. Therefore, not only the yellow senescent
577 maize leaves but also the dry brown leaves, which remain attached to the plant, are an important

source of BVOCs. By comparing cumulative BVOC emissions from senescent maize leaves with those of artificially senescing species of the Poaceae family, it was found that maize leaves were clearly among the strongest emitting species during that leaf developmental stage. Nevertheless, an improved comparison of cumulative BVOC emissions among senescent species of the Poaceae family might benefit from additional measurements on naturally senescing plants instead of results obtained from cutting and drying experiments.

Whereas senescent leaves showed a large diversity of emitted compounds, BVOC emission rates for young, semi-mature, and mature leaves 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 patterns among the different leaf developmental stages. The contributions from the young-growing, mature, and senescent stages to the total methanol emission from a typical leaf of a maize plant were estimated to be 61, 13, and 26%, respectively.

Although our growth chamber study provided new information on the contribution of senescence and other leaf developmental stages to the BVOC emissions from maize, additional studies, preferably in field conditions and at a wide variety of meteorological conditions, are definitely required to better constrain BVOC emissions for this important C4 crop species for use in regional and global atmospheric chemistry and climate models.

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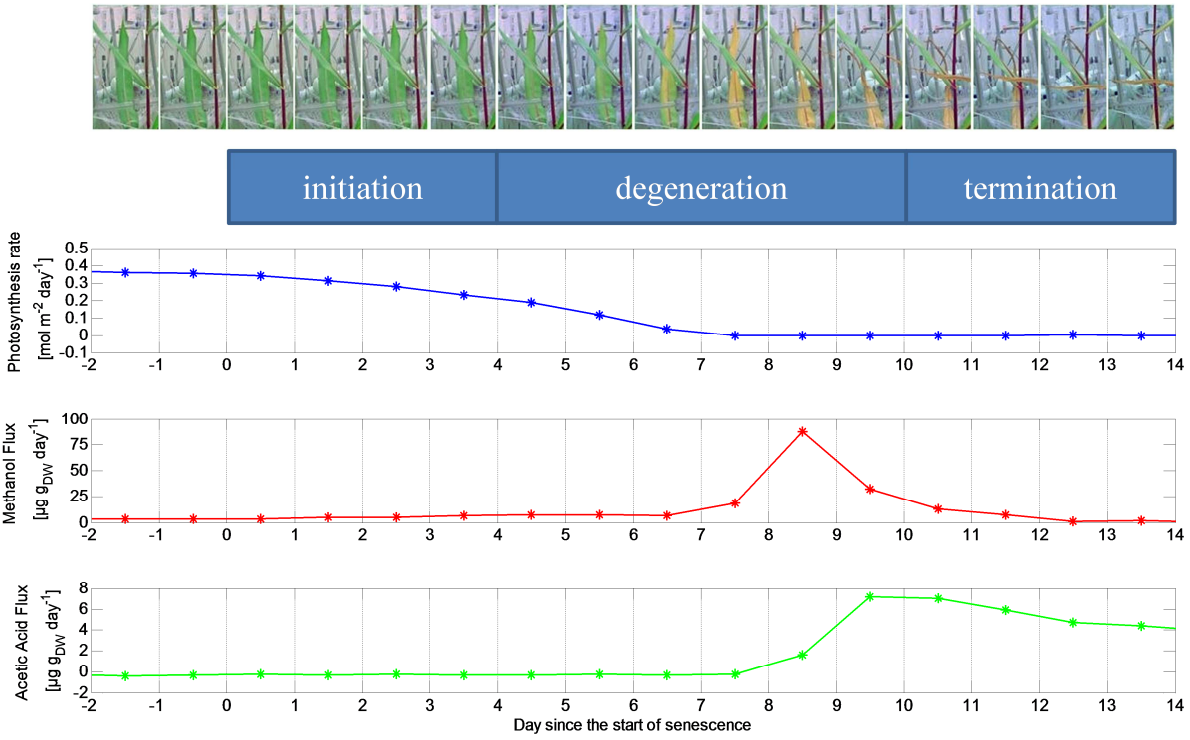
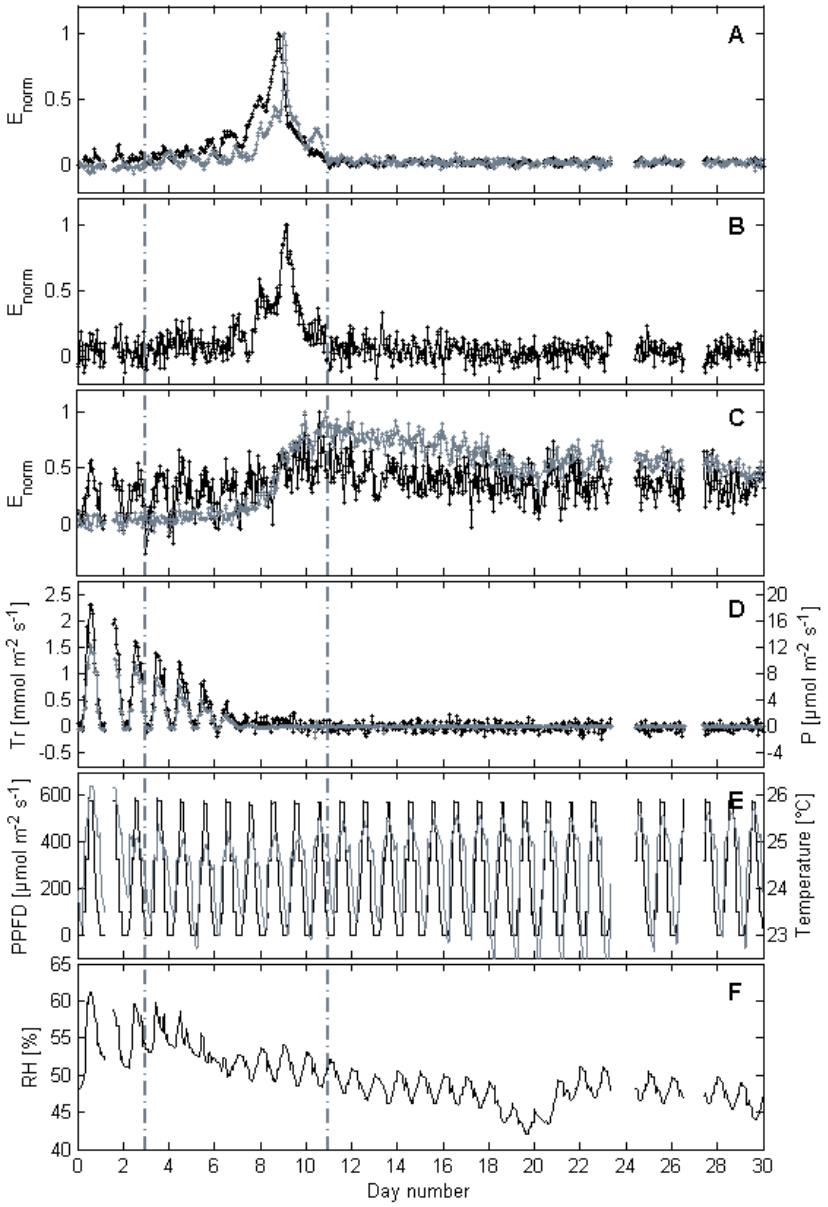
Figure 1


initiation
degeneration
termination
Photosynthesis rate [mol m-2 day-1]
Methanol Flux [µg gDW day-1]
Acetic Acid Flux [µg gDW day-1]
Day since the start of senescence

827

Figure 2



828

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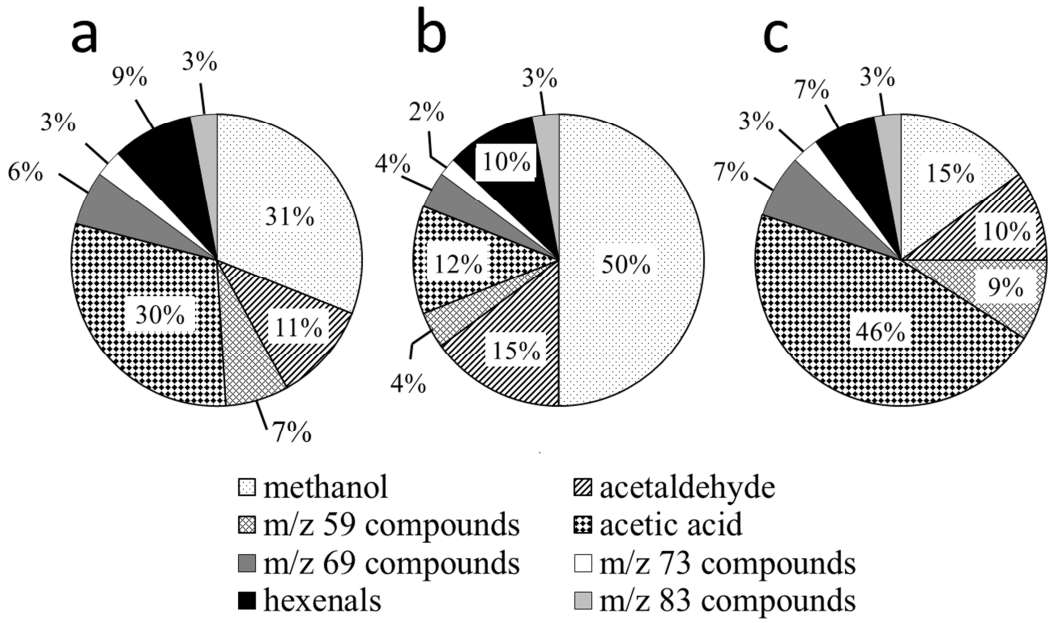
Figure 3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3%
methanol
m/z 59 compounds
m/z 69 compounds
hexenals
acetaldehyde
acetic acid
m/z 73 compounds
m/z 83 compounds

Figure captions

Fig. 1: Temporal evolution of the daily photosynthesis rate and the daily emission rates of methanol and acetic acid for a specific senescent leaf and corresponding pictures of the leaf taken daily at 10 AM. The different phases of developmental leaf senescence are indicated.

Fig. 2: Normalised instantaneous emission rates of methanol (A, black dots), acetaldehyde (A, grey dots), hexenals (B), m/z 59 compounds (C, black dots), and acetic acid (C, grey dots) from a senescent maize leaf since the onset of senescence, together with the transpiration rate (Tr) (D, black dots) , the photosynthesis rate (P) (D, grey dots), light (PPFD) (E, black line), temperature (T) (E, grey line) and relative humidity (RH) (F) conditions in the enclosure. The BVOC emission rates have been normalised 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 of the degradation (day 3) and termination (day 11) phase of senescence, respectively. Gaps in the data are due to instrument failure.

Fig. 3: Proportion of cumulative BVOC emissions from the senescent leaves for different periods: (a) from the onset of leaf senescence to the end of the investigations, (b) during the leaf chlorosis period (degeneration phase), (c) from the end of the chlorosis period to the end of the measurements (termination phase). Cumulative emissions were averaged over 10 replicates and only compounds which individually made up over 0.5% of the total BVOC emission were considered. Total cumulative BVOC emissions for a, b and c were 0.67 ± 0.11, 0.33 ± 0.04 and 0.34 ± 0.08 mg/g_{DW}^{-1}, respectively.