

"Influence of environmental factors on
biosphere-atmosphere exchange of
carbonyl sulfide (OCS) with special focus on
elevated CO₂-levels and soils"

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Summary

Carbonyl sulfide (OCS) is the most abundant sulfur containing trace gas in the troposphere. It is linked to the carbon and sulfur cycle and, acts as a greenhouse gas in the troposphere and is related to the stratospheric aerosol layer. It is also considered a possible tracer for CO₂ in Gross primary productivity (GPP), because there is a close link between the uptake of OCS and exchange of CO₂ between plants and atmosphere. Both soils and plants play an important role in the budget of OCS. Therefore, any estimation of GPP based on the OCS exchange must take into account the contribution of the exchange with the corresponding soil. Many environmental factors influence soil-atmosphere or plant-atmosphere OCS exchange. In this work the influence of the ambient CO₂ and OCS mixing ratio, soil moisture and antimicrobial agents on this exchange has been examined.

The OCS exchange of four arable soils was examined at elevated CO₂ mixing ratios (soil pore concentrations) at varying soil moisture. These arable soils showed a tendency towards OCS emission at low and high soil moisture and towards OCS uptake at medium soil moisture. With increasing CO₂ mixing ratio, three soils showed an increase of OCS emission at high and low soil moisture and a reduction of OCS uptake at medium soil moisture. One organically fertilized soil showed an increase in OCS uptake at medium soil moisture with increasing CO₂ mixing ratio instead. Treatment with chloroform vapor yielded ambivalent results but demonstrated the involvement of biotic processes in soil-atmosphere OCS exchange for those soils. Application of Nystatin and Streptomycin solutions to one arable soil and one rainforest soil demonstrated that fungi possibly dominated OCS uptake in those soils.

In another set of experiments OCS exchange of three forest soils and one arable soil at high and low OCS mixing ratio at varying soil moistures was measured. Based on these measurements OCS production (P_{OCS}) and consumption (U_{OCS}) at 1000 ppt OCS mixing ratio were determined. Their compensation points were found to vary with soil moisture but generally indicated that needle forest soils mainly act as sinks. Compensation point variations calculated based on these process

studies were in good agreement with single values as found in the literature. Furthermore, the OCS exchange measured with one forest soil sample (soil from the SMEAR II station in Hyytiälä, Finland) agreed very well with field data from the same site as published recently by other researchers.

OCS uptake of plants at elevated CO₂ mixing ratios was examined in a third set of experiments. OCS uptake declined with increasing CO₂ mixing ratios. Calculations of stomatal conductance based on the water vapor released by the examined plants demonstrate that the reduction of OCS uptake at higher OCS concentration is mediated by stomatal aperture. This control over OCS uptake by stomatal conductance is in good agreement with literature. An experiment with a toxin causing a stomatal opening further confirmed that mechanism.

Zusammenfassung

Carbonylsulfid (OCS) ist das häufigste schwefelhaltige Spurengas in der Troposphäre. Es ist Bestandteil des Schwefel- und Kohlenstoffkreislaufs, agiert als Treibhausgas in der Troposphäre und trägt zur stratosphärischen Aerosolschicht bei. Es wird außerdem als möglicher Tracer für CO₂ in Betrachtungen zur Bruttoprimärproduktion (Gross Primary Production, GPP) diskutiert, weil eine enge Verbindung zwischen OCS Aufnahme und CO₂ Austausch durch Pflanzen besteht. Sowohl Pflanzen als auch Böden spielen eine wichtige Rolle im OCS Budget. Deshalb kann man eine GPP-Abschätzung nicht anstellen, ohne die Rolle der jeweiligen Böden in die Berechnung mit einzubeziehen. Eine Vielzahl von Umweltfaktoren beeinflussen den OCS Austausch sowohl zwischen Pflanzen und Atmosphäre als auch Böden und Atmosphäre. In dieser Arbeit wird der Einfluss des atmosphärischen Mischungsverhältnisses von CO₂ und OCS, der Bodenfeuchte und der Einfluss von antimikrobiellen Agentien auf diesen Austausch untersucht.

Der OCS Austausch von vier landwirtschaftlichen Böden wurde bei verschiedenen Bodenfeuchten und unter erhöhtem CO₂ Mischungsverhältnis untersucht. Diese Böden zeigten eine Tendenz zur OCS Emission bei hohen und niedrigen Bodenfeuchten und eine Tendenz zur OCS Aufnahme bei mittleren Bodenfeuchten. Mit zunehmendem CO₂ Mischungsverhältnis zeigten drei der Böden eine Zunahme der OCS Emission bei hoher und niedriger Bodenfeuchte und eine Abnahme der OCS Aufnahme bei mittlerer Bodenfeuchte. Ein mit Kompost gedüngter Boden zeigte stattdessen zunehmende OCS Aufnahme mit steigendem CO₂ Mischungsverhältnis bei mittlerer Bodenfeuchte. Eine Behandlung mit Chloroform erbrachte uneindeutige Resultate, demonstrierte jedoch die Beteiligung biotischer Prozesse am OCS Austausch dieser Böden. Der Einsatz von Nystatin und Streptomycin Lösungen bei einem landwirtschaftlichen Boden und einem Regenwaldboden zeigte, dass in diesen beiden Böden Pilze möglicherweise die dominanten OCS Konsumenten sind.

In weiteren Experimenten wurde der OCS Austausch von drei Waldböden und einem landwirtschaftlichen Boden unter hohem und niedrigem OCS Mischungsverhältnis bei verschiedenen Bodenfeuchten gemessen. Auf diesen Messungen basierend wurden die OCS Produktion (P_{OCS}) und Aufnahme (U_{OCS}) bei 1000 ppt OCS Mischungsverhältnis. Der OCS Kompensationspunkt variiert in Abhängigkeit von der Bodenfeuchte und weist generell auf die Funktion von Nadelwaldböden als OCS – Senke hin. Die Variation des Verlaufes der Kompensationspunkte erklärt sehr gut die Lage von Einzelmessungen früherer Arbeiten. Besonders betont werden soll, dass der OCS Austausch einer Bodenprobe (Nadelwaldboden bei der SMEAR II Station in Hyytiälä, Finnland) zeigte eine sehr gute Übereinstimmung mit Feldmessungen die kürzlich von einer anderen Forschungsgruppe publiziert wurden.

Die OCS Aufnahme von Pflanzen unter erhöhten CO_2 Mischungsverhältnissen wurde in einem dritten Satz von Experimenten bestimmt. Die Aufnahme von OCS nahm mit steigendem CO_2 Mischungsverhältnis ab. Berechnungen der stomatären Leitfähigkeit basierend auf dem von den Pflanzen abgegebenem Wasserdampf zeigten, dass diese Abnahme der OCS Aufnahme durch Reduktion der Öffnungsweite der Stomata bedingt ist. Ein Versuch mit einem Pilztoxin, das ein Öffnen der Stomata bewirkt, bestätigte diesen Zusammenhang zusätzlich.

Data usage and contribution statement

The overall strategy and topic of the biosphere-atmosphere OCS exchange project and this dissertation was suggested and supervised by Prof. Dr. Kesselmeier.

The work presented here is part of a large cooperation examining the influence of environmental factors on biosphere-atmosphere OCS exchange. There are three groups of experiments. During the time the presented work was created, Prof. Dr. Kesselmeier, Prof. Dr. Yi and Dr. Behrendt were involved in the cooperation.

The first group of experiments examines the influence of elevated CO₂ mixing ratios on soil-atmosphere exchange. Additionally, it is examined whether fungi or bacteria are the main contributors to soil-atmosphere OCS exchange.

Soil-atmosphere exchange of 4 soils has been measured as described in Section 2.4

- at 5 different CO₂ mixing ratios

Soil-atmosphere exchange of 4 soils has been measured as described in Section 2.4

- at 5 different CO₂ mixing ratios after chloroform vapor treatment

Soil-atmosphere exchange of 2 soils has been measured as described in Section 2.4

- at one CO₂ mixing ratio after nystatin or streptomycin treatment

Experiments of this first group were planned by me. I performed 60% of the measurements. 40% of the measurements were performed by a lab assistant under my instruction. All data processing, calculations, interpretation and evaluation of the results were done by me. Prof. Dr. Kesselmeier, Prof. Dr. Yi and Dr. Behrendt provided helpful comments, and suggestions. The calculation of soil moistures was aided by an Excel template provided by Dr. Behrendt. Dr. Behrendt constructed the automated soil chamber system used to perform the measurements and instructed me on the handling of the soil chamber system.

The second group examines the influence of ambient OCS mixing ratios on soil-atmosphere OCS exchange.

Soil-atmosphere exchange of 4 soils has been measured as described in section 2.4

- at two different OCS mixing ratios

Soil-atmosphere exchange of 1 soil (2 subsample groups, “dry” and “field fresh”) has been measured as described in chapter 2.4

- at 3 different OCS mixing ratios

Experiments the second group were planned by Prof. Dr. Yi. I was involved in the conceptual stage and instructed Prof. Dr. Yi on the handling of the OCS Analyzer. Furthermore, I performed parts of the sample preparation. A first initial data processing and calculation were performed by Prof. Dr. Yi. At this stage, I took over the complete work and performed recalculations and continuing work. Summarizing, I did 70 to 80% of the data interpretation and evaluation. Prof. Dr. Kesselmeier, Prof. Dr. Yi and Dr. Behrendt provided helpful comments, and suggestions.

Both groups of experiments also examine the influence of soil moisture on soil-atmosphere OCS exchange.

The third group examines the influence of elevated OCS on plant-atmosphere OCS exchange.

- The OCS uptake by four tree species and one annual C4 plant at different CO₂ mixing ratios was measured using a dynamic chamber system. Due to the secondary nature to the project and limitations in time plant exchange data could only partially be processed and evaluated. The plant data and entailing discussion presented here is therefore to be understood as an outlook and marked accordingly.

I planned and performed all plant measurements. I did all data processing and calculations. I did the data evaluation and interpretation. Prof. Dr. Kesselmeier provided helpful comments, instructions and suggestions.

Results of the first group of experiments were published in JGR: Biogeosciences with me as first author:

Bunk, R., Behrendt, T., Yi, Z., Andreae, M. O., & Kesselmeier, J. (2017). Exchange of carbonyl sulfide (OCS) between soils and atmosphere under various CO₂ concentrations. *Journal of Geophysical Research: Biogeosciences*.

Results of the second group have been included in a manuscript to be submitted to Biogeosciences. A shared first authorship between me and prof. Dr. Yi is intended. The manuscript is in the final stage of preparation. Tentative title: Carbonyl sulfide (OCS) exchange between soils and the atmosphere under the control of soil moisture and compensation points

A manuscript covering the third topic is planned.

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1 Introduction

1.1 Properties and budget of carbonyl sulfide

Carbonyl sulfide (OCS) is a sulfur-containing trace gas in the atmosphere. With a Henri Constant of $2.0 \times 10^{-4} \text{ mol m}^{-3} \text{ Pa}^{-1}$ (Sander 2015) the solubility of OCS in water is rather low. With an estimated lifetime of 1.5 to 3 years in the troposphere (Montzka et al 2007), it is the sulfur containing trace gas with the longest life time (Andreae and Ferek, 1992). The average tropospheric mixing ratio is 476 ppt (Montzka et al., 2007) in the Northern Hemisphere. Due to its longevity, it can be transported to the stratosphere where it is photochemically transformed and contributes significantly to the sulfate aerosol layer (Crutzen 1976; Brühl et al., 2012; Sheng et al., 2015). This makes carbonyl sulfide an important link between the sulfur cycle and the sulfate aerosol layer in the stratosphere. It has been estimated to provide about 30% of the sulfate of that layer (Brühl et al., 2012). In the troposphere, OCS contributes to radiative forcing and thus to global warming, but the cooling effect of light backscatter by its contribution to the stratospheric aerosol is about the same size, so cooling and warming contributions of OCS are cancelling each other out (Brühl et al., 2012).

The OCS mixing ratio shows clear seasonality in both the Northern and Southern Hemisphere (Montzka et al. 2007). This seasonality is driven by terrestrial vegetation and its corresponding vegetation period in the Northern Hemisphere while Southern hemisphere seasonality is driven by ocean processes (Kettle et al., 2002).

Oceanic emissions are considered the dominant OCS source while terrestrial vegetation is the primary sink (Kettle et al., 2002). Plant OCS exchange will be further discussed in Section 1.3. Minor sources include biomass burning, rayon production, volcanic activity and anoxic soils (Kremser et al., 2016, Kettle et al., 2002). Other sinks are transport to the stratosphere,

photolysis, and oxidative reactions with the hydroxyl ion (OH⁻) or oxygen radical (O[·]) (Kremser et al., 2016; Kettle et al., 2002). Traditionally, oxic soils are considered sinks for OCS while anoxic soils are considered sinks (Watts et al., 2000; Kettle et al., 2002) but environmental factors can influence a soils OCS exchange behavior, in some cases enough to induce a shift from sink to source or vice versa. Exchange behavior of soils will be discussed further in Section 1.2.

The budget of OCS is currently not balanced (see Figure 1) with current estimations of sinks exceeding estimated sources (Kremser et al. 2015). However, variation between yearly averages is low (Montzka et al., 2007, Kremser et al., 2016). This implies that currently not all source and sink strengths are properly estimated. Figure 1 shows an overview of current estimates of OCS sinks and sources in the troposphere by Kremser et al. (2015).

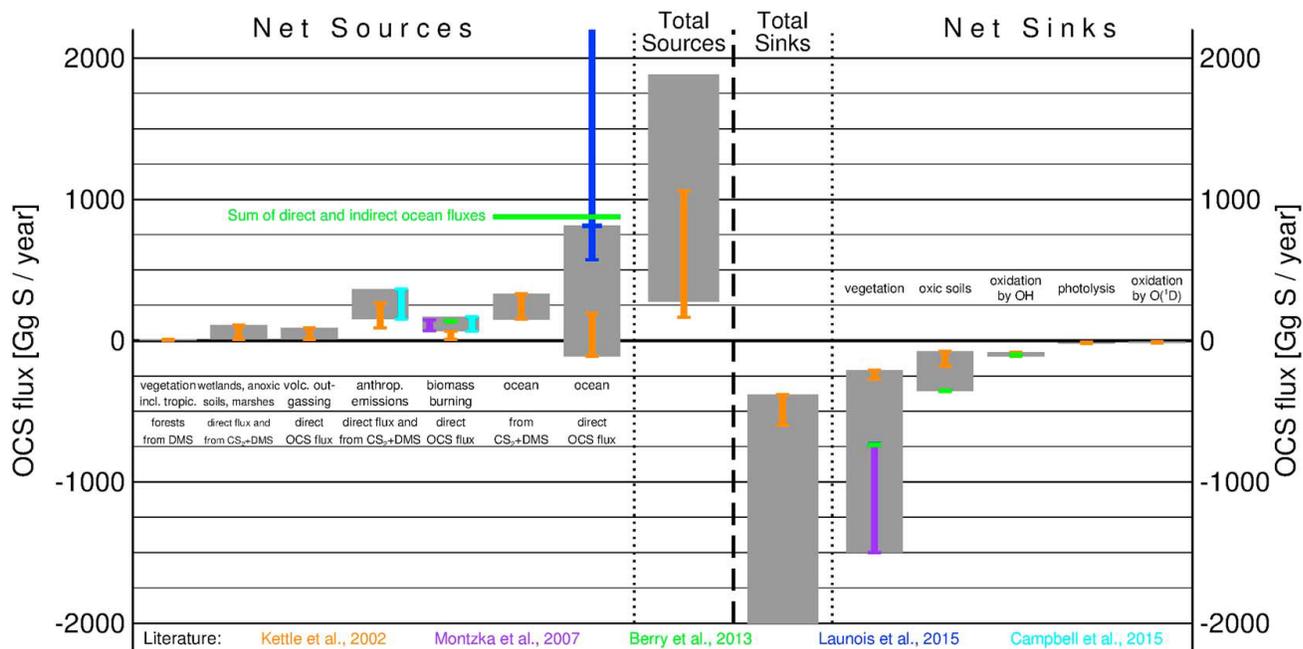


Figure 1 (figure, including caption, from Kremser et al., 2016) Tropospheric OCS budget as represented in the current literature. The grey bars show the realistic ranges for the different source and sink terms as well as the total sources and sinks. Also included are individual source and sink estimates by Kettle et al. [2002] (orange), Montzka et al. [2007] (purple), Berry et al. [2013] (green), Launois et al. [2015] (blue), and Campbell et al. [2015] (cyan). The OCS sink terms due to chemical reactions go back to the study by Chin and Davis [1993] and comprise both tropospheric and stratospheric losses. Later, the same authors estimated the total stratospheric OCS loss to 30 Gg S/yr with a 71% contribution from photolysis, 22% from reaction with O(1D), and 7% from reaction with OH [Chin and Davis, 1995]. This increases the chemical loss terms slightly, in particular for photolysis.

It has been suggested that underestimated ocean emissions from the tropic zone might fill the gap between sources and sinks (Berry et al., 2013) but a very recent study by Lennartz et al. (2017) has shown that this is likely not the case (Lennartz et al., 2017). A recent study by Du et al. (2016) showed that OCS emission from domestic coal fires has been severely underestimated. The corrected estimates add about 30 Gg sulfur per year to the sources solely for China. If this applies in similar fashion for other countries, the gap between sources and sinks might be reduced.

It has been shown that phosphoenolpyruvate carboxylase (PEPCO), ribulose-1,5-bisphosphate-carboxylase-oxygenase (RubisCO), and carbonic anhydrase (CA) consume OCS. (Protoschill-Krebs and Kesselmeier, 1992; Protoschill-Krebs et al., 1996; Blezinger et al., 2000; Sandoval-Soto et al., 2005). CA irreversibly cleaves OCS into H₂S and CO₂ (Protoschill-Krebs et al., 1996). The product of OCS uptake by RubisCO is 1-thio-3-phosphoglycerate (Lorimer and Pierce 1989). It is important to note that all three of these enzymes are key enzymes for CO₂ uptake as this implies a tight link between biosphere-atmosphere exchange of CO₂ and OCS. The relevance of this will be discussed in chapter 1.4. Furthermore, Ogawa et al. (2013) identified an enzyme with high similarities to CA in *Thiobacillus thioparus* strain THI 115, which has similar OCS degrading abilities as CA but much lower reactivity with CO₂. In addition to PEPCO, RubisCO, COSase, and CA, another enzyme using OCS as substrate is nitrogenase (Seefeldt et al., 1995).

1.2 Soil-atmosphere OCS exchange

The role of soils in OCS exchange is ambiguous. Based on early experiments (Adams et al., 1981; Aneja et al., 1979a; Aneja et al., 1979b), soils were originally considered to be major source for OCS (Khalil and Rasmussen, 1984; Andreae and Jaeschke, 1992; Chin and Davis, 1993; Johnson et al., 1993). This was contradicted by later field experiments that demonstrated soils to act as OCS sinks (Castro and Galloway, 1991; Fried et al., 1993; DeMello and Hines, 1994; Kuhn et al., 1999,

Simmons et al., 1999; Yi et al., 2007; Sun et al., 2017). This discrepancy was caused because earlier work used sulfur free flushing air as a flushing gas for their chamber measurement (Castro and Galloway, 1991, DeMello and Hines, 1994; Kesselmeier et al., 1999). Because OCS uptake scales with ambient OCS mixing ratio (Lehmann and Conrad 1996; Kesselmeier et al., 1999), the use of sulfur free flushing air caused an underestimation of (potential) OCS uptake (Watts et al., 2000). Other environmental factors that have been shown to influence OCS exchange by soil are soil moisture (Kesselmeier et al., 1999; Van Diest and Kesselmeier, 2008, Liu et al., 2007), temperature (Goldan et al., 1987; Lehmann and Conrad, 1996; Kesselmeier et al., 1999, Liu et al., 2007), redox value (Devai and Delaune, 1995) and light (Whelan and Rew, 2015). By now, it is accepted that soils may act as sinks or sources, depending on their properties and environmental factors (Watts et al., 2000; Kettle et al., 2002; Montzka et al., 2007).

While abiotic uptake processes like hydrolysis in the soil water are possible, soil OCS uptake is generally thought to be mainly biotic. Enzymatic uptake of OCS in soils or by various soil bacteria has been shown (Lehmann and Conrad, 1996; Kesselmeier et al., 1999; Kato et al., 2008; Van Diest and Kesselmeier, 2008 Ogawa et al., 2013). Additionally, OCS degradation by one strain of the soil fungus *Fusarium solani* has also been shown (Li et al., 2010). This is not surprising, as CA is a ubiquitous enzyme found in all organisms (Yonemura et al., 2005) and the presence of CA in soil has been shown (Seibt et al., 2006; Wingate et al., 2008). Taking into account different activation/activities of CA, Ogée et al. (2016) developed a model to predict OCS uptake by soils. The presence of RubisCO in soil has also been reported (Selesi et al., 2007; Videmšek et al., 2009; Salinero et al., 2009; KoilRaj et al., 2012; Nowak et al., 2015; Liu et al., 2016). On the other hand, two enzymatic pathways for OCS production have been reported: production from thiocyanate by thiocyanate hydrolase (Katayama et al., 1992; Katayama et al., 1998) and production from carbon disulfide by carbon disulfide hydrolase (Smith and Kelly, 1988, Smeulders et al., 2011). Lehmann and Conrad (1996) also observed an increase of OCS emission from soils after the addition of thiocyanate. Thiocyanate is expected to be found in soils because it is produced by glucosidase from mustard oil (glucosinolates) when plant cells become damaged (Wood 1975, Bones and Rossiter 2006, Halkier and Gershenzon 2006, Morant et al., 2008). Consequently, OCS exchange by soils must be seen as the sum of biotic consumption and production. Likely, abiotic

processes also contribute in most cases. Because uptake strength is, among other factors, driven by the ambient OCS mixing ratio, this implies the existence of a compensation point, the mixing ratio where a soils production (P_{OCS}) and Consumption (U_{OCS}) are equal and their net exchange (J) becomes zero (Lehmann and Conrad, 1996, Kesselmeier et al., 1999). Part of the research presented in here was aimed at a better understanding of this compensation point and its relationship with soil properties and soil moisture. Another part of the experiments aimed at a better constraint on the biotic fraction of OCS exchange by arable soils utilizing agents inhibiting microorganisms.

1.3 Plant atmosphere OCS exchange

Plants are widely recognized as the main OCS sink. OCS is enzymatically cleaved or integrated by enzymes found in all plants (Lorimer and Pierce, 1989; Protoschill-Krebs and Kesselmeier, 1992; Protoschill-Krebs et al., 1996; Blezinger et al., 2000; Sandoval-Soto et al., 2005). Application of ^{35}S labelled OCS resulted in an uptake of the sulphur isotope and its integration into amino acids (Brown et al., 1986). Disregarding small emission numbers which may be understood as measurement errors, there are almost no reports of direct OCS emission from plants. Two noteworthy exceptions are emissions from fungi infected oilseed rape reported by Bloem et al. (2012) and by a salt march plant (*Batis maritima*) reported by Whelan et al. 2013. No production mechanisms in plants have been reported so far. Therefore, transport of OCS between plants and atmosphere is expected to be unidirectional. Because plants take up trace gases through their stomata and the sink for OCS are the enzymes within the leaf, OCS uptake by vegetation is controlled by stomatal conductance (Goldan et al., 1988; Watts et al., 2000; Yonamura et al., 2005; Sandoval-Soto et al., 2005). While obviously controlled by stomatal conductance, there is indication that plant OCS uptake is principally light independent, as uptake of OCS in the dark after incomplete closure of stomata (Yonamura et al., 2005) or by vegetation that lack stomatal regulation (Kuhn and Kesselmeier, 2000) has been reported.

1.4 Links in CO₂ and OCS uptake, ambient CO₂ mixing ratios and biosphere-atmosphere OCS exchange

In early work about OCS exchange Goldan et al. (1988) found that the uptake resistance for OCS was very similar to that of CO₂. Given this high similarity in uptake resistance, Goldan et al. (1988) suggested that the uptake ratio of these two gases should be equal to the ratio of their respective atmospheric mixing ratios of about 1.3×10^{-6} . This first quantitative link between OCS uptake and terrestrial primary productivity was further developed by Chin and Davis (1993). Based on the aforementioned discoveries regarding OCS uptake via the key enzymes of assimilation by Protoschill-Krebs and Kesselmeier (1992), Chin and Davis (1993) used a ratio of deposition velocities for CO₂ and OCS of 1 to quantify the uptake of OCS by terrestrial vegetation from the atmospheric ratios of these two atmospheric gases, effectively linking the terrestrial uptake of OCS to the net photosynthetic production (NPP) of an ecosystem. To include the preferred uptake of OCS over CO₂ at leaf and enzyme level (Kesselmeier and Merk, 1993; Protoschill-Krebs et al., 1996), Sandoval-Soto et al. (2005) recalculated the ratios for most major ecotypes, taking into account the deposition velocities of OCS and CO₂, and found them almost exclusively to be greater than 1. Furthermore, they concluded that calculations of OCS uptake should not be based on NPP but on gross primary production (GPP) instead, as the uptake of OCS into vegetation is unidirectional and OCS is irreversibly split into H₂S and CO₂ within plants. This model was confirmed by Campbell et al. (2008), who demonstrated that such a GPP driven model fits the OCS modeled data much better to the measured values.

There is an increasing interest now in exploiting the close relationship between OCS and CO₂ uptake to use OCS as a proxy for the estimation of ecosystem GPP (Asaf et al., 2013; Berry et al., 2013; Berkelhammer et al., 2014). The potential, limitations, and requirements of this prospect are discussed in detail in a review by Wohlfahrt et al. (2012). Regarding the unidirectional uptake of OCS by plants and its use as a GPP proxy within an ecosystem, one of the largest question

marks is the role of other flux contributors, which might result in a complex mixture of exchange processes and limit its usefulness as a CO₂ proxy.

Atmospheric CO₂ concentrations have been rising ever since the industrial revolution, and are expected to keep going up in the future. This will affect the CO₂ uptake by vegetation, but also the exchange of OCS. As OCS and CO₂ are binding to the same active center of CA (Notni et al., 2007), RubisCO (Lorimer and Pierce, 1989), and possibly also PEPCO, it can be assumed that these substrates are in competition with each other. Little is known about the development of the OCS sink under elevated CO₂. Stimler et al. (2010) saw no cross sensitivity between CO₂ and OCS during their measurements with various C3 plants, but there are a few reports about a decrease in the OCS uptake under elevated CO₂ (White et al., 2010; Sandoval-Soto et al., 2012). As the typical CO₂ concentrations in soils are much higher than those tested in previous experiments with plants, the potential of competitive inhibition may be much stronger in soils. Furthermore, an increase of the activity of bacteria by higher CO₂ mixing ratios as reported by several authors (Dehority 1971; Repaske and Clayton 1978; Samuelov et al., 1991) might boost bacterial OCS production or consumption. In this case, the capacity of soils to act as OCS sink would become difficult to predict. Part of the first group of experiments presented here aimed to examine the impact of CO₂ concentration on the OCS exchange. Exchange rates for five soils under five different CO₂ concentrations were measured using an automated dynamic chamber system. According to the literature, soil CO₂ concentrations can easily reach thousands of ppm even in the upper cm of soils, with CO₂ concentration usually increasing with depth (e.g., Gerstenhauer, 1972; Kiefer and Amey, 1992 and literature cited therein; Hirano et al., 2003; Yonemura et al., 2009; Sakurai et al., 2015). In close accordance with these reports, mixing ratios of up to 7600 ppm were chosen, the upper limit for the OCS instrument used for the presented measurements. The first group of soil-atmosphere exchange experiments aimed to improve the understanding on how soil moisture and atmospheric CO₂ concentration influence the OCS exchange of soils and whether biotic processes are involved in this exchange.

Though the CO₂ mixing ratio, plants are typically exposed to, is smaller than the average soil pore CO₂ mixing ratio, plant OCS exchange might be impacted in a similar fashion. Therefore, plant-

atmosphere OCS exchange at elevated CO₂ mixing ratios was examined using a dynamic plant enclosure (cuvette) system in the third group of experiments presented here.

1.5 Aim and motivation

Carbonyl sulfide is an important trace gas in the atmosphere. It plays an important role in the radiative balance of the earth and is connected to the sulfur and carbon cycle. Furthermore, it is a promising tool for better constraining ecosystem GPP (gross primary productivity). However, the current budget of OCS is not balanced with indications that either sources are under- or sinks overestimated. If the contribution to the OCS exchange between forests and the atmosphere by soils is unclear, an estimation of GPP based on OCS deposition will not be more reliable than the estimation based on direct CO₂ exchange measurements taking into account net uptake and respiration rates. A number of environmental factors can influence the biosphere-atmosphere exchange of OCS. Not all of the exchange processes and the influence of environmental factors on these exchange processes are currently well understood, as indicated by the imbalanced budget. The aim of the work presented in here was to improve the understanding of

- (1) The influence of elevated CO₂ on soil-atmosphere and plant-atmosphere OCS exchange affecting OCS production and/or consumption in microbes and plants as well as on stomatal conductance
- (2) The OCS compensation points of soils and how they define the role of soils in the OCS budget
- (3) The influence of soil moisture on soil OCS exchange in conjunction with the above factors

2. Materials and Methods

2.1 Characterization of the OCS analyzer

Carbonyl sulfide concentrations were measured with a new laser based instrument (LGR OCS/CO₂ analyzer model 907 0028, Los Gatos Research, USA). Because this analyzer was the centerpiece of all experiments presented here, special care was taken to characterize the performance and limitations of the instrument. The LGR OCS analyzer uses cavity-enhanced absorption-spectroscopy techniques (Off-Axis Integrated-Cavity Output Spectroscopy) where the laser wavelength is scanning selected absorption features of the target species. The measured absorption spectra recorded are corrected according to cell temperature and pressure and effective path length, and is supposed to deliver a quantitative measurement of mixing ratios without external calibration. However, the calibration was checked and it was found that the OCS signal of the analyzer was strongly affected by the water vapor content of the measured air. This caused especially severe problems when the water content fluctuated because of evaporation or transpiration. A strong underestimation of OCS concentrations mimicked a spurious uptake. After initially noticing this effect in measurements and some simple tests, it was confirmed using a Nafion dryer (Model Perma Pure MD™-110, Perma Pure LLC, USA) in reverse mode (as suggested by LGR). A gas sample of known OCS concentration was humidified in steps by adding water vapor with a Dew Point generator to the flushing air in the outer tube of the Nafion dryer. Increasing water vapor caused a significant drop in the recorded OCS concentration, exceeding any possible dilution effect. The impact of the water vapor concentration on the apparent OCS concentration is shown in Figure 2. This bias is normally corrected by an algorithm and other colleagues working with this model report no such problems (i.e., *Berkelhammer et al.*, 2014; *Belviso et al.*, 2016). The problem may be individual to this instrument, which was one of the first ones built and was also adapted for measurements at high CO₂ concentrations. Therefore, all gas samples were dried by a Nafion dryer (Model Perma Pure MD™-110, Perma Pure LLC, USA) before entering the OCS analyzer. Dry compressed air was used for flushing of the outer tube. Under these conditions the

determination of OCS was sufficiently accurate and precise (see Table 1). Additionally, the analyzer was tested at the Forschungszentrum Jülich (FZJ, Germany) by measuring defined concentrations of OCS produced with a setup using permeation tubes. In addition, at low concentrations (ppt range) a NOAA Standard (449.8 ± 1.4 ppt, Essex stainless steel cylinder, cylinder number SX-3584, NOAA, USA) was measured. Measured and calculated OCS concentrations did fit very well for higher concentrations. Comparison to the NOAA standard showed an average underestimation of 7%. Maximal underestimation was 10%. Consequently, measured fluxes might be underestimated by 7%.

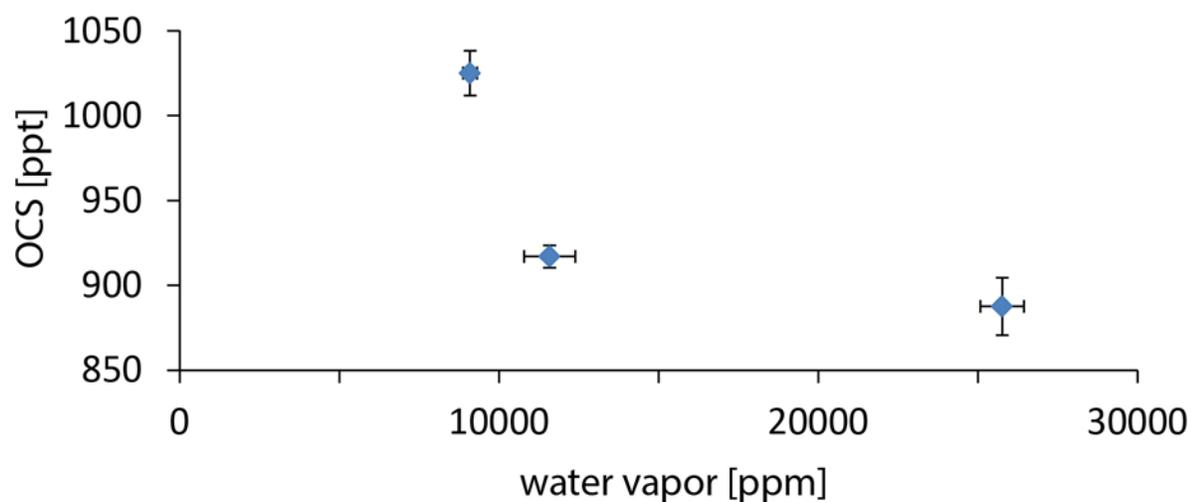


Figure 2 Apparent OCS mixing ratio measured at three different water vapor concentrations. The same sample gas was humidified using a Nafion dryer in reverse mode (transferring water vapor through the Nafion mesh from the flushing air which had a higher water vapor concentration than the gas sample in the inner tube)

Table 1 Calculated and measured amount of CO₂ and OCS in calibration mixtures with increasing CO₂

CO ₂ concentration (ppm)			OCS concentration (ppt)		
Calculated	measured ± SD	%	Calculated	measured ± SD	%
499	469 ± 1.01	94	533	539 ± 3.87	101
998	904 ± 2.58	91	533	534 ± 3.27	100
2988	2738 ± 10.1	92	532	544 ± 4.07	102
4970	4476 ± 4.97	90	531	531 ± 4.16	100
6945	6255 ± 6.55	90	530	526 ± 6.45	99
8911	7919 ± 4.88	89	528	515 ± 8.81	97

To test for a possible interference of the Nafion dryer, OCS (500 ppb OCS in nitrogen, Air Liquide, Germany) was added to a dry air stream consisting of CO₂ in synthetic air (100 ppm, Air Liquide, Germany). One-half was directly sent to the analyzer, the other half after passing a Nafion dryer. A three-way valve (stainless steel, SS-45XS12, Swagelok, USA) was used to switch between the sample streams. Measurements of OCS were not affected by the use of a Nafion dryer as shown in Figure 3.

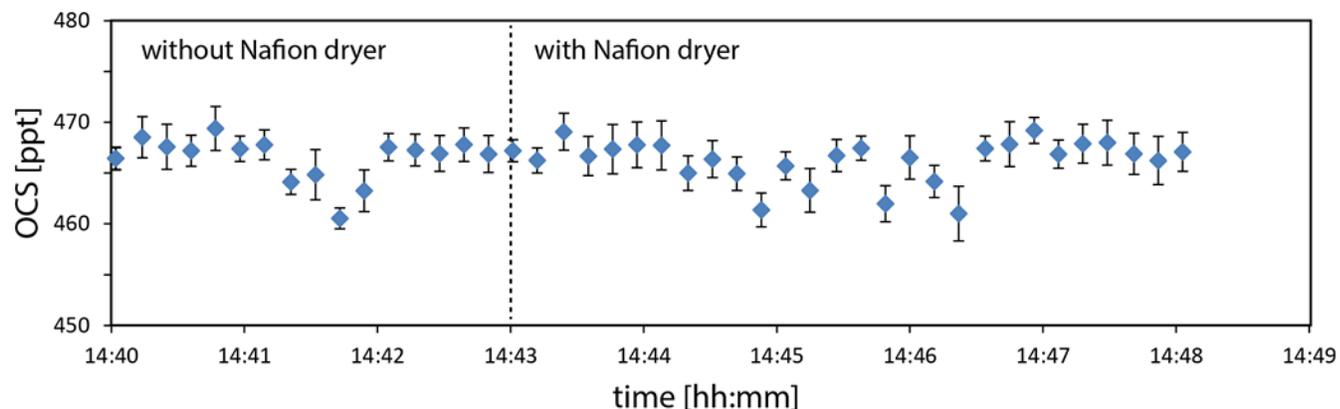


Figure 3 Measured OCS concentration (blue diamonds) in dry air with (right) and without (left) passing a Nafion dryer. Error bars denote the measurement uncertainty of the OCS/CO-Analyzer.

The precision was found to be better than 5 ppt, and the detection limit as defined a three times SD of the standard noise was around 35 ppt.

The influence (bias) of high CO₂ levels on the determination of OCS was tested by measurements with synthetic air, containing defined amounts of OCS and CO₂. A basic flow of 10 l min⁻¹ of synthetic air was flushed through three glass bottles (5 l volume each, HWS Labortechnik, Germany) that were used as mixing devices and were equipped with a ring poppet valve (stainless steel, SS-4C-5, Swagelok, USA) opening at an overpressure of 35 kPa (5 psi) for safety reasons. Increasing amounts of pure CO₂ were added with flow rates between 5 and 90 ml min⁻¹ via a mass flow controller (MKS). OCS was added from a cylinder with a compressed and certified gas mixture of OCS in nitrogen with a flow rate of 9.6 ml min⁻¹. An open junction just before the analyzer prevented overpressure within the instruments. The data from the first five minutes after the start of gas flow were discarded to account for an equilibration of the system, and the following 10-minute average was used. The OCS and CO₂ concentrations are reported as calculated and measured values (Table 1). The recovery of OCS appears quantitative within experimental uncertainties under all conditions and matches the calculated values. There is a slight trend towards underestimation (3 %) at higher CO₂, but the values remain within the error ranges of the mass flow controllers. The determination of CO₂ is accurate enough at normal concentrations, but an underestimation with increasing CO₂ was observed, which however did not affect the measurements or interpretations.

2.2 Soil samples

Soils can be very heterogeneous in structure, mineral composition and microbial activities. Therefore, all soil samples investigated were thoroughly sieved with a stainless steel sieve with a mesh size of 2 mm (mineral soils) or 16 mm (organic soils) and were stored in polyethylene bags at 5°C until analyzed and mixed in order to obtain a homogenous sample, which allowed a subsequent and highly reproducible sample withdrawal over time. Fresh subsamples of arable soil in Mainz were air dried for comparison. To minimize microbial adaptations, the samples were stored in the refrigerator at 5 °C to prevent (or slow down) any further development of microbial communities and nutrient content. This well-accepted approach allowed an incubation of soil samples starting at a comparable developmental stage at each step. No incubated soil sample was used twice. Experiments with an automated soil laboratory allowed us to measure 4-5 samples simultaneously against an empty chamber (see Section 2.4). Hence, the different soil types were investigated simultaneously under identical conditions, delivering consistent data sets covering the conditions from 100% maximum water holding capacity (MWHC) to dryness. When measurements came to an end and soils samples reached a dry condition, samples were exchanged against new ones for the stepwise incubation under different CO₂ (soils in Section 2.2.1) or OCS (soils in Section 2.2.2) regimes.

A very close agreement was found when measuring a few samples from the same sample soil pool again under similar conditions. Mainz soil had been measured both for the first group of experiments and later again for the second group under similar conditions. Figure 4 shows the replicated OCS exchange as observed under 440 ppm CO₂ and close to 1000 ppt OCS mixing ratio. This performance of the Mainz arable soil compares well with that from a few months earlier. Conditions (cycle time, flushing rate, temperature) for the first second group of experiments differed slightly from the measurements in the first group.

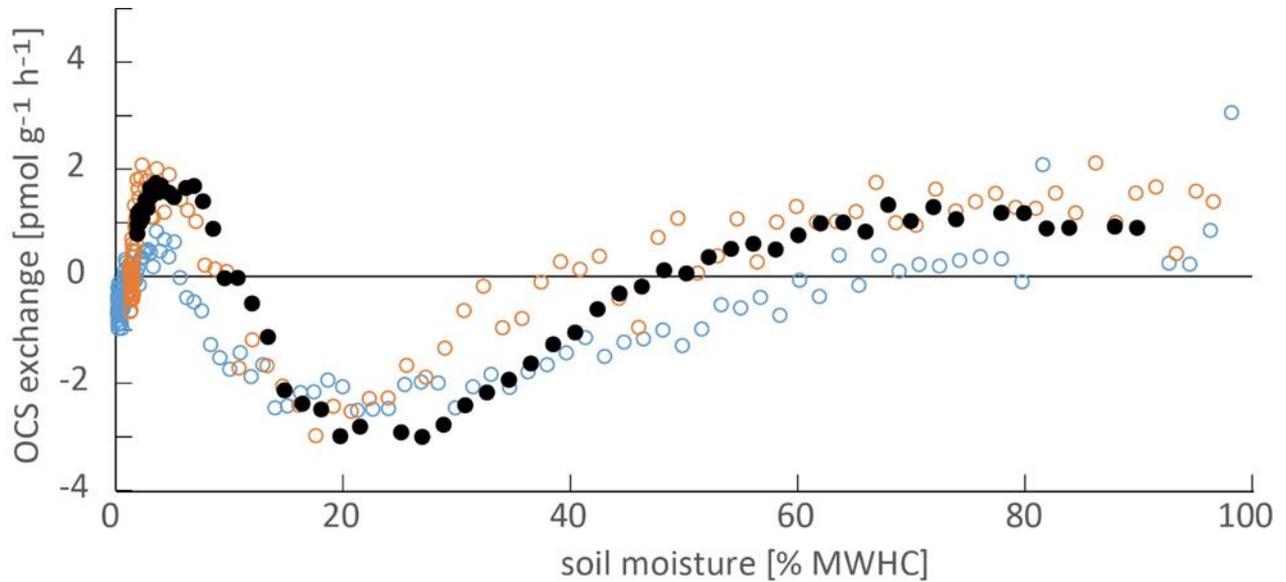


Figure 4: Carbonyl sulfide exchange for arable Mainz soil in two measurements in April 2014, (empty orange and empty blue circles) and one measurement in September 2014 (full black circles) at similar conditions. Soil moisture is given as percent of the soils maximum water holding capacity (MWHC)

Soil characteristics were determined by an external company (Envilytix, Wiesbaden, Germany) and are summarized in Table 2 and 3. Some values of soils that were used in other recent works have been taken from either Behrendt et al. (2014) or Oswald et al. (2015) and are marked accordingly in Table 3.

Table 2: Properties of the investigated soil samples in the first group of experiments (PD is Particle Density, LOI is Loss on Ignition).

Sample	NH ₄ ⁺ [mg kg ⁻¹]	NO ₂ ⁻ [mg kg ⁻¹]	NO ₃ ⁻ [mg kg ⁻¹]	SO ₄ ²⁻ [mg kg ⁻¹]	PO ₄ ³⁻ [mg kg ⁻¹]	C [%]	N [%]	S [%]	pH	LOI [%]	PD [g cm ⁻³]
Mainz	<0.05	0.07	3,78	65.0	0.05	2.5	0.17	0.03	7.6	3.7	2.6
Nördlingen corn	<0.1	0.01	86.0	2.58	5.94	1.6	0.21	0.03	7.1	5.1	2.6
Nördlingen sugar beet	1.6	0.17	75.6	17.56	10.72	1.6	0.20	0.04	7.2	4.9	2.6
Himalaya	1.9	0.03	402.8	22.26	112.76	3.0	0.33	0.06	7.4	8.7	2.5
Suriname	68.9	<0,01	360.6	47.18	0.42	8.7	0.56	0.07	3.9	18.3	2.3

Table 3: Properties of the investigated soil samples in the second group of experiments. a: the data is from Behrendt et al. (2014) and b: the data is from Oswald et al. (2015)

Sample	Ecosystem	NH ₄ -N	NO ₃ -N	TC	TN	TS	pH	MWHC
		[mg/kg]	[mg/kg]	[%]	[%]	[%]	-	[g g ⁻¹]
Mainz fresh	Wheat field	<0.04	0.85	2.454	0.166	0.028	7.64	0.668
Mainz dry	Wheat field	1.45	0.87	2.445	0.158	0.020	7.48	0.668
Waldstein blueberry	Spruce forest	239.6 ^a	36.9 ^a	40.18	1.894	0.255	3.2 ^a	3.788
Waldstein spruce	Spruce forest	982.6 ^a	90.2 ^a	45.27	2.059	0.263	3.0 ^a	3.681
Finland scots pine forest	Scots pine forest	1.6 ^b	2.0 ^b	47.56	1.37	0.173	3.0 ^b	8.234

2.2.1 Soil samples for measurements at elevated CO₂ or application of inhibiting agents

For this group of experiments, five different soils were used: (1) “Mainz soil”, an arable soil collected 2014 from a wheat field near Mainz-Finthen (49.95°N 8.25°E), Germany. Samples from this site have been collected and investigated already earlier (Kesselmeier et al., 1999; Van Diest et al., 2008). (2) “Nördlingen sugar beet”, an arable soil collected in April 2014 from an organically fertilized sugar beet field at the Nördlinger Ries, Germany, near Nördlingen (48.8883333°N 10.5358333°E). (3) “Nördlingen corn”, an arable soil collected in April 2014 from a conventionally fertilized corn field at the Nördlinger Ries (48.8883333°N 10.5358333°E). (4) “Suriname”, a tropical rainforest soil collected (05.0763°N, -55.0029°E) in Suriname in 2012. (5) “Himalaya”, an arable soil collected from a rice field (30.83480°N, 76.98631°E) in the Himalaya in 2014. Table 4 summarizes the experimental conditions for measurements with soils of section 2.2.1

Table 4 Experiments with elevated CO₂ or with inhibiting agents performed for soils in section 2.2.1. The OCS||CO₂ column shows the average (whole experiment) OCS and CO₂ mixing ratios of OCS and CO₂. The SD of the average OCS mixing ratio was about 10 ppt (whole experiment), while the SD of the average CO₂ mixing ratio was more variable and is given in the bracket behind the mixing ratio. The chamber temperature was 20 °C.

untreated soils					
OCS [ppt] CO₂[ppm] concentration	Mainz	Nördlingen corn	Nördlingen sugar beet	Himalaya	Suriname
490 440(20)	x	x	x	x	x
490 990(20)	x	no sample	no sample	no sample	x
490 2000(20)	x	x	x	x	x
490 3850(20)	x	x	x	x	x
490 7600(70)	x	x	x	x	x

chloroform sterilized soils					
OCS [ppt] CO₂[ppm] concentration	Mainz	Nördlingen corn	Nördlingen sugar beet	Himalaya	Suriname
490 470(20)	x	x	x	x	x
490 1010(10)	x	x	no sample	no sample	x
490 2000(10)	x	x	x	no sample	x
490 4030(30)	x	x	x	no sample	x
490 7520(30)	x	x	x	x	x

antifungal or antibiotic Mainz or Suriname			
OCS [ppt] CO₂[ppm] concentration	untreated	Nystatin	streptomycin
925 430(10)	x	X	x

2.2.2 Soil samples for measurement at varying OCS concentrations

For this group of experiments, soil samples from four sites were used: (1) an arable soil in Mainz, Germany with wheat planted before, the site near the one Kesselmeier et al. (1999) and Van Diest and Kesselmeier (2008) studied (49.95°N 8.25°E), (2) an organic layer (Oh) originated from an understory dominated by blueberries, (50.1420°N 11.8665°E), (3) an organic layer (Oh) originated from a young spruce understory, (50.1425°N 11.8673°E). Both, (2) and (3) are located

within a spruce forest, Waldstein, Germany. (4) a Scots pine litter layer from the Hyytiälä site, Finland (61.846°N 24.295°E, sampled in July 2012). Table 5 summarizes the experimental conditions of measurements performed with soils from section 2.2.2

Table 5 Experiments with elevated OCS performed for soils in section 2.2.2. The OCS || CO₂ column shows the average (whole experiment) OCS and CO₂ mixing ratios of OCS and CO₂. The SD of the average OCS and CO₂ mixing ratio is given in the bracket behind the mixing ratio. The chamber temperature was 20 °C.

soils at elevated OCS					
OCS [ppt] CO₂[ppm] concentration	Mainz, dried	Mainz, fresh	Waldstein, blue berry	Waldstein, spruce	Finland, spruce
50(5) 420(10)	x	x	x	x	x
500(10) 420(10)	x	x	not measured	not measured	not measured
1000(5) 420(10)	x	x	x	x	x

2.3 Plants

All plants were kept in a greenhouse. The temperature in the greenhouse was 15 °C in the winter and up to 35 °C in the summer. Lighting followed the natural light conditions outside the greenhouse. Under experimental conditions plants and cuvettes were incubated inside a plant growth chamber under day and night cycles of 13 hours/11 hours with a maximum of 1400 PAR [$\mu\text{mol m}^{-2} \text{s}^{-1}$] over the day and a chamber temperature of 23 °C. From 2014 to mid-2015 plants were watered with tap water by an automated system, applying a fixed daily amount that could be manipulated by adjusting the outlet. However, a fixed daily amount of water, even if regulated for seasonal changes, proved inadequate for good plant health and the automated commercial system showed a high vulnerability to malfunctions due to calcium carbonate deposits. Therefore, starting mid-2015, plants were watered manually when their soil felt dry to the touch. Instead of a fixed amount of water, water was added until the speed with which the soil took up water slowed. This resulted in a rhythm of daily watering in summer and approximately every

third day in winter. Plant health improved significantly following this change in watering policy. Infection by white flies was also reduced. *Quercus robur* trees were treated with the broadband fungicide Duaxo (Compo, Germany) during spring and summer thrice a week. *Fagus sylvatica* and *Sorbus aria* were treated once a week.

Trees were trimmed occasionally to fit into the plant cuvette. After trimming, trees were always given an adjustment period of at least 3 days before being used for measurements.

After transfer into the plant cuvette for measurements, plants were allowed an adjustment period of at least one day before start of the measurements.

Three to five-year-old *Quercus ilex* trees were purchased from Burncoose & South Down Nursery, UK and potted in 28 cm pots with “Floregard” soil (Floraself, Germany). They were grown for 2 to 3 years in the MPIC greenhouse prior to the exchange measurements presented.

Corn (*Zea mays*, “Badischer Gelber”, Kiepenkerl, Germany) was grown from seeds in spring 2015 and 2016 in a commercial soil mix “Saat- und Anzuchterde” (Neudorff, German).

Quercus robur, *Fagus sylvatica*, and *Sorbus aria* trees were bought as two-year-old seedlings from a local nursery (Darmstädter Forstbaumschulen GMBH, Germany). They were potted in 28 cm pots in commercial soil “Floragard” soil (Floraself, Germany) and grown for one year in the MPIC greenhouse. Despite treatment, *Quercus robur* trees were almost constantly infected by mildew during the vegetation period. Only individuals with less than 5% visual coverage were used for measurements. They were grown for 1 to 2 years in the MPIC greenhouse prior to the exchange measurements presented.

2.4 Experimental setup, soil measurements

This section covers the experimental setup for the soil-atmosphere OCS exchange measurements.

All soil measurements were performed using an automated soil chamber system established in its current form by Behrendt et al. (2014). The OCS Analyzer described above was integrated into the analyzer unit of this automated chamber system. In short, this automated soil chamber system consists of four main units: (1) the gas dilution unit, which generates the air with desired qualities (relative humidity, trace gas concentrations) for flushing the soil chambers, (2) the thermostat valve unit, regulating the flow of flushing air through the soil chambers and selecting from which chamber the air is fed to the analyzer unit by computer controlled Galtec valves (Entegris, Billerica, model no. 203-3414-215), (3) the thermostat cabinet unit, housing up to seven Plexiglas chambers and regulating their temperature, and (4) the analyzer unit, consisting of the chosen set of analyzers. The setup allows connecting various analyzers, tailored to the needs of the individual experiment. In this case an NDIR (nondispersive infrared) CO₂/H₂O analyzer (LiCOR 840A, LiCOR Inc., USA) and an OCS/CO₂ analyzer (see Section 2.1) were used. A flushing air stream with the desired concentration of water vapor, OCS and CO₂ was generated in the gas dilution unit, then directed by the thermostat valve unit into the thermostat cabinet unit. There, the cuvettes (five samples and one empty reference) were flushed with this air stream at set rates. Samples were drawn by the analyzer units to determine the trace gas concentrations.

Only the dynamic mode described in Behrendt et al. (2014) was used. A cylinder of compressed gas (500 ppb OCS in nitrogen, Air Liquide, Germany) and a cylinder of pure CO₂ (4.5, Air Liquide, Germany) were connected via two Mass Flow Controllers (MKS Instruments, Germany) to the system, allowing the addition of controlled amounts of OCS and CO₂ to the flushing air stream. All experiments within the cabinet unit were performed at 20 °C chamber temperature.

In the first group, there were three sets of experiments: “natural, non-sterilized”, “CHCl₃ sterilized” and “antibacterial and antifungal inhibition”. The procedures for each set are described below. To reach 100 % WHC the soils were wetted with milliQ-water (18 MΩ, ELGA-Purelab ultra ionic, Vivendi Water Systems Ltd., United Kingdom) to reach field capacity. The maximum water holding capacity was determined with the filter method described in Behrendt et al. (2014) using Whatman filter paper no. 42.

Cuvettes were flushed with a total flow of 2.4 l min⁻¹ of compressed air purified by a pure-air generator (PAG 003, Ecophysics, Switzerland). To this flow, OCS and CO₂ were added via mass flow controllers to reach the desired mixing ratios. Source gases were compressed pure CO₂ (4.5, Air Liquide, Germany) and OCS, 500 ppb in nitrogen (Air Liquide, Germany).

“Natural non-sterilized soils”:

Samples of 60 g soil (except for the Suriname soil with 20 g) were put into the chamber and wetted to the maximum water holding capacity. Chambers were flushed with dry air, containing 490 ppt OCS and either 440(±20), 990(±20), 2010(±20), 3850(±30) or 7600(±70) ppm CO₂. The basis for the flushing air was compressed, dry ambient air. The pure air generator did not fully remove background CO₂, resulting in small fluctuations in the CO₂ mixing ratio. These changes happened gradually over time, usually not more than 5 ppm per hour. Above, the average for the whole experiment is given, with its standard deviation (±SD) in brackets. To better reflect these gradual changes, reference concentrations between sets of measurements were interpolated as described in Section 2.6. Trace gas concentrations in five sample chambers and one empty reference chamber were measured in a repeating sequence, while the samples were gradually dried out by the dry flushing air. When water evaporation ceased, the air-dried samples were weighed and fully dried at 105 °C for 24 hours, then weighed again to determine the dry weight.

“CHCl₃ fumigated”:

Chloroform fumigation affects fungi, bacteria and protozoans in soil samples. While protozoans are killed completely, usually a small portion of fungal and bacterial populations remain active. Furthermore, fungi are less sensitive than bacteria (Ingham and Horton, 1987). 60 g of sample (20 g in case of the Suriname soil) were exposed to the vapor of 300 µl chloroform for 48 hours in a desiccator. For the 470 ppm experiment, the exposure time was 24 hours and 200 µl chloroform were used. After fumigation with CHCl₃ the samples were wetted and incubated as described for “natural” measurements. As described under “natural non-sterilized soils” above, CO₂ mixing ratios varied slightly resulting in 470(±20), 1010(±10), 2000(±10), 4030(±30), 7520(±30) ppm. Due to the lack of sufficient soil, no CHCl₃ fumigated experiments could be performed for the “Himalaya” soil at 1010, 2000, and 4030 ppm CO₂, as well as for “Nördlingen sugar beet” soil at 1010 ppm CO₂.

“Treatment with antifungal and antibiotic compounds”:

Streptomycin is an antibiotic that acts against Gram-negative and Gram-positive bacteria by binding to the 30S ribosomal subunit. At the concentration used (200 µg L⁻¹), streptomycin is usually not a potent agent against fungi, requiring roughly a thousand-fold concentration of streptomycin (3.5-8 mg ml⁻¹) to be effective (Robinson et al., 1944, Robinson, 1946). Nystatin is mainly active against most fungi, while bacteria are not affected (Lampen et al., 1957). It acts by binding to the membranes of sensitive organisms making them permeable for cations (Kinsky, 1962). Five samples each of Mainz soil and Suriname soil were treated with nystatin or streptomycin, or left untreated. Measurements were performed at 925 ppt OCS and 430 (±15) ppm CO₂ in the flushing air. Nystatin and streptomycin inhibition was performed in the course of the wetting procedure by adding 100 µg L⁻¹ nystatin solution or 200 µg L⁻¹ streptomycin solution instead of pure water.

In the second group of experiments (see Section 2.2.2), soil chambers were filled with either 80 g of Mainz soil (mineral, Ap layer) or 20 g of the organic layers of three forest soils, and then the soils were wetted to nearly 100 % MWHC with deionized water ($R > 18 \text{ M}\Omega$). One chamber remained empty for reference. Chambers were flushed with about 2.5 l min^{-1} of dry compressed air that had been passed through a pure air generator (PAG 003, Ecophysics, Switzerland) beforehand. OCS and CO_2 mixing ratios in the inlet flushing gas were adjusted to about 400 ppm for CO_2 and 50, 500, or 1000 ppt for OCS by addition of those gasses in respective amounts by mass flow controller from standard gas cylinders (1% CO_2 , Westfalen, Germany) and (500 ppb OCS, Air Liquide, Germany). Samples were drawn by the analyzer units from the outlet of individual chambers to determine the trace gas mixing ratios (including OCS [LGR OCS/ CO Analyzer, Los Gatos Research, USA], CO_2 , H_2O [Licor 860, LiCOR, USA]).

2.5 Experimental setup, plant measurements

This section covers the experimental setup of the plant-atmosphere OCS exchange measurements.

Plant-atmosphere OCS exchange was measured using a dynamic chamber setup. The enclosures, further referred to as cuvettes, were effectively bags of chemically inert FEP Teflon foil on a supportive frame. Each cuvette had an effective volume of approximately 70 Liters. Cuvettes were flushed with compressed air at rates of 17 to 22 l min^{-1} . The flushing rate was controlled by mass flow controllers (MFC) (MKS, Germany).

The air inside the cuvettes was thoroughly mixed by a Teflon coated propeller near the top of the cuvette and the air stream of the outlet tube placed near the bottom of the cuvette. Cuvettes were kept at a minimal overpressure (below 0.1 bar but above 0 bar in excess of ambient

pressure) by regulating the tightness of the bottom binding, guaranteeing the flow through any potential leak to be directed outwards of the cuvette.

Cuvettes were housed in climate chamber (VB 1014 Bioline, Weiss Umwelttechnik GMBH, Germany) ensuring constant temperature of 23 °C (leaf temperature increased to 27 °C in the light due to light absorption) and constant light (1350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR). The climate chambers can also regulate the water vapor mixing ratio, but because the interior of the cuvettes inside the climate chamber was isolated from the climate chambers air, humidity inside the cuvettes was determined by the water vapor mixing ratio of the flushing air. After passing a glass wool filter, the flushing air was humidified to about 7000 to 9000 ppm water vapor mixing ratio in a humidification bottle. The humidification bottle is a custom made 5 l airtight glass bottle, filled to one fourth with glass spheres (2 cm diameter) and water (up to 3 l). The dry compressed air was inserted at the bottom of the bottle and bubbled through the liquid water, taking up water vapor in the process. A second, empty bottle served as a trap for any potential liquid water. After the water trap the humidified flushing air was adjusted to the desired OCS and CO₂ mixing ratios by addition of CO₂ or OCS from standard gas cylinders (500 ppb OCS, Air Liquide, Germany and pure CO₂, Air Liquide, Germany) via MFC.

Samples were drawn by Pumps (KNF Neuberger, Freiburg, Typ N86KTDC) to the OCS-Analyzer (see Section 2.1 for analyzer details) and a LiCOR 7000, a commercially available CO₂/water vapor instrument based on non-dispersive infrared (NDIR) light absorption. All parts of the pumps in contact with the sample gas are covered by Teflon. Sample air to be measured by the OCS analyzer was dried by a Nafion dryer (Model Perma Pure MD™-110, Perma Pure LLC, USA) to avoid water vapor induced bias (see Section 2.1). Leaf temperature were measured with type E thermocouples (Omega, Germany). Figure 5 shows a photograph of one of the used cuvettes and a simplified schematic of the experimental setup for the plant-atmosphere OCS exchange measurements.

Because the OCS Analyzer has only one measurement channel, the inlet was switched between the sample and reference cuvette in 10 minute intervals. The gap between two reference or sample intervals was filled by linear interpolation as described in Section 2.6.

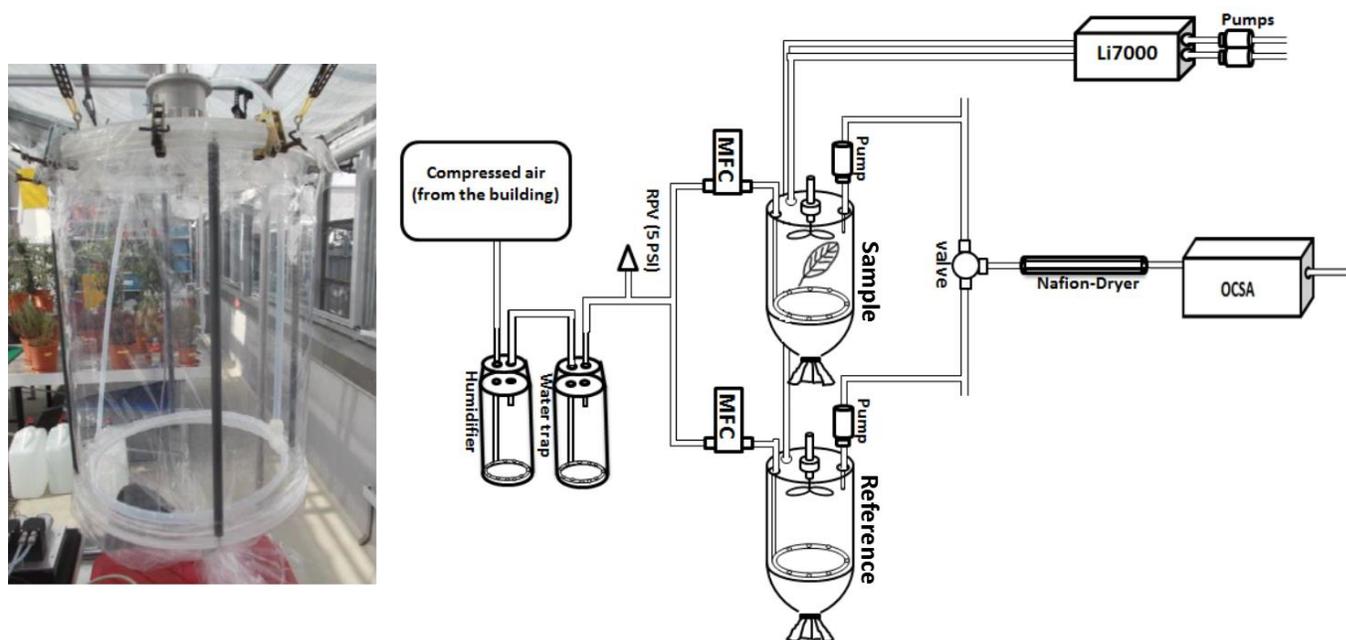


Figure 5 Photography of the used cuvette (right) and a simplified schematic representation of the experimental set up (left). The Mass flow controller (MFC) that were used to add OCS or CO₂ was placed between the water trap and the ring poppet valve and is not shown in the schematic. Symbols are not true to scale.

Because some researchers have reported OCS emission from Teflon chambers or Teflon foil (private communication), the Teflon foil used to construct the cuvettes in the presented experiments was tested for possible OCS emissions. Compressed ambient air was split in two air streams of 17 l each, the lowest cuvette flushing rate used in conjunction with the plant cuvettes. A Galtek three-way valve (Entegris, USA) was used to alternate between the two flows. One of them was fed directly to the valve, the other after passing through a cuvette. The retention time in the cuvette, based on flow size and cuvette volume, was four minutes. Figure 6 shows the measured OCS mixing ratio in the flushing air stream with (valve position 1) and without (valve

position 0) passing through a cuvette. No difference in OCS mixing ratio was observed with or without passage through the cuvette, indicating there was no significant amount of OCS emission by the used Teflon foil at the flow rates chosen for the presented experiments.

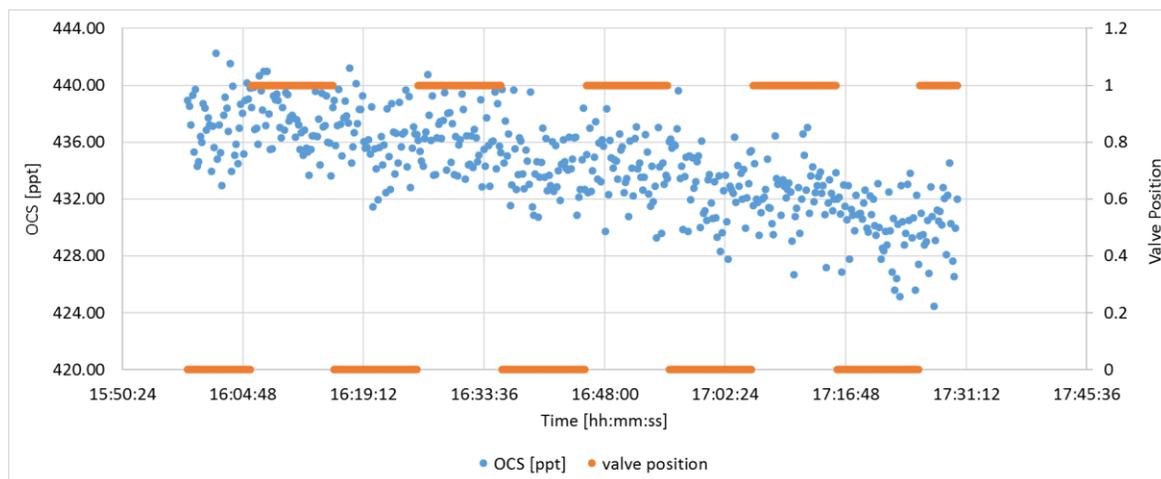


Figure 6 Measured OCS mixing ratio measured directly from the flushing air or after passing one of the cuvettes used in the plant-atmosphere exchange measurements. Air directly from the flushing air stream without passing the cuvettes (valve position 0) or from an empty cuvette (valve position 1) was fed to the analyzer in 10 minute intervals. No alteration of the OCS mixing ratio by the cuvette was measurable.

In this third group of measurements, there were two sets of experiments. In the first set, exchange of OCS between plants and the atmosphere were measured. Stem and leaves of *Quercus ilex* (holm oak), *Quercus robur* (European oak), *Sorbus aria* (whitebeam), *Fagus sylvatica* (beech) or *Zea mays* (corn) were placed in the sample cuvette. Cuvettes were closed at the bottom with bindings; the tightness adjusted to a degree that let excess air escape from the cuvette but kept a minimal overpressure in the cuvettes. Soil and pot of the sample plant were outside the cuvette and thus not contributing to the measured trace gas mixing ratios inside the cuvettes. Gas samples from the sample cuvette and the reference cuvette were drawn by pumps and analyzed for their trace gas concentrations as described above. The desired CO₂ mixing ratio in the flushing air was set by adjusting the amount of pure CO₂ added to the flushing air. After a continuous CO₂ mixing ratio was reached, trace gas exchange was measured for 30 to 40 minutes,

then the next CO₂ concentration was set. The process was repeated until trace gas exchange at all desired CO₂ mixing ratios had been measured. For *Quercus robur*, trace gas exchange at each CO₂ mixing ratio was measured for one day instead. Water vapor mixing concentrations were measured with a LiCOR 7000, OCS and CO₂ mixing ratios were measured with the OCS analyzer described in Section 2.1. OCS uptake (F_{CO_2}), Transpiration (E) and stomatal conductance (GH₂O) were calculated from the difference in OCS concentration and water vapor concentration as described in Section 2.6.

For the second set of experiments, a branch of *Quercus robur* was cut under water and was placed in a nutrient solution (0.1M KCl; 0.01M NaCl; 0.01M CaCl solution). The branch cut was placed in the sample cuvette and trace gas exchange measured analogous to the measurements with whole plants above at mixing ratios of 520 to 540 ppt OCS and 400 ppm CO₂, respectively. After 6 hours of measurement, the light in the climate chamber was turned off. After a dark period of twelve hours the light was turned back on. Then, following Turner and Gratini 1969, the nutrient solution was replaced by an identical nutrient solution, to which 1.5×10^{-5} M Fusicoccin (Sigma Aldrich, Germany) and 0.075% (v/v) ethanol (Roth, Germany) had been added. Fusicoccin is a fungal toxin that induces stomatal opening (Clauson-Kaas et al., 1944; Turner and Gratini 1969; Squire and Mansfield, 1972). After 12 hours, the light was turned off again. Trace gas exchange behavior in the light and dark before and after Fusicoccin application is compared.

2.6 Calculations

Continuous changes of the gravimetric soil moisture in soil samples were calculated according to Behrendt et al. (2014), based on the calculation of the mass balance for evaporated water, which was monitored by a LiCOR 840A, thus getting a continuous track of soil water content. Soil

moisture (SM), given in percent of maximum water holding capacity (MWHC), was derived from the gravimetric soil moisture according to Eq. (1).

$$SM = \frac{\theta_{t_i}}{\theta_{max}} * 100 \quad (1)$$

where θ_{t_i} is the gravimetric soil moisture at any given time, θ_{max} is the gravimetric soil moisture directly after wetting, and SWC is the soil water content in percent of the respective soil's maximum water holding capacity. Soil moisture of the Finland soil was recalculated into $m^3 m^{-3}$ for comparison with recently published field data (Sun et al., 2017) using the density of water at 20° C (0.998 g cm^{-3}) and the bulk density of that soil (0.1 g cm^{-3}) reported in Pumpanen and Ilvesniemi 2005.

Exchange rates, J [$\text{pmol g}^{-1} \text{ h}^{-1}$], were calculated based on the chamber flushing rates, Q [mol h^{-1}], the dry weight of the soil sample, (m_{soil} [g]), and the difference of OCS concentration between sample and reference chamber (OCS_s [pmol mol^{-1}]- OCS_r [pmol mol^{-1}]) following Eq. (2).

$$J = \frac{(OCS_s - OCS_r) * Q}{m_{soil}} \quad (2)$$

Additionally exchange rates $J_{,A}$ [$\text{pmol m}^{-2} \text{ s}^{-1}$] were calculated according to Equation (3).

$$J_{,A} = \Delta_{OCS} \times \frac{Q}{A_c} \quad (3)$$

Where Δ_{OCS} [pmol mol^{-1}] is the difference in OCS concentration between the sample and reference chamber, Q [mol s^{-1}] is the flushing rate, and A [m^2] is the sample chamber area.

The OCS/CO₂ analyzer, like most instruments of its kind, has only one measurement channel. Therefore, the trace gas concentrations in the air from the dynamic chambers were measured in sequential cycles. Each cycle started with the reference chamber, followed by the sample chambers. Each chamber was measured for 10 minutes. Thus, one measurement cycle lasted 50 to 60 minutes, depending on how many sample chambers were used. The reference data for a given time, t_i , was calculated from the measured reference data by linear interpolation according to Eq. (4).

$$f(t_i) = f_0 * \frac{t_1 - t}{t_1 - t_0} + f_1 * \frac{t - t_0}{t_1 - t_0} \quad (4)$$

Here $f(t_i)$ is the trace gas concentration at time t_i , and f_0 is the trace gas concentration at the last reference measurement before t_i , while f_1 is the trace gas concentration at the reference measurement following t_i , and t_0 is the time at which f_0 was measured. Finally, t_1 is the time at which f_1 was measured.

To account for disturbances when switching from one chamber to the next, only those 150 seconds (of the 600 seconds) of a measurement period per chamber were used when the fluxes through the chamber had stabilized. The first 390 seconds after switching to another chamber and the last 60 seconds before switching were ignored. With this procedure, the fluctuations of the OCS concentration in the reference chambers' flushing air were less than 1 %.

High, optimal and low moisture ranges were defined qualitatively based on the correlation of OCS exchange rates and soil moisture, placing the border between the three ranges at the intersection of OCS exchange with the x-axis (compromising between all CO₂ concentration sets) as illustrated in Figure 7. These exchange rates were fitted with the multi-peak curve-fitting tool

of Origin Pro 9 and each moisture range integrated by the trapezoid integration approach according to Eq. (5):

$$A = (MWHC_d - MWHC_w) * \frac{J_{MWHC_w} + J_{MWHC_d}}{2} \quad (5)$$

where A is the area of a given trapezoid, $MWHC_d$ is the soil water content at the drier point, $MWHC_w$ is the soil water content at the wetter point, J_{MWHC_w} is the emission rate corresponding to $MWHC_w$, and J_{MWHC_d} is the emission rate corresponding to $MWHC_d$. Accordingly, the total integrated OCS exchange for each soil moisture range is the sum of all trapezoids within its borders.

OCS compensation points (CP) are the OCS mixing ratio at a given soil moisture at which OCS uptake (U_{OCS}) and production (P_{OCS}) are balanced and the net exchange (exchange rate, E_{OCS}) is zero. CP can be calculated by the following process: The exchange rate, J at a given soil moisture can be expressed as

$$J = P_{OCS} + U_{OCS} \quad (6)$$

where P_{OCS} is the OCS production, U_{OCS} the OCS uptake. P_{OCS} for any ambient OCS mixing ratio will be equal to the net exchange rate at ambient OCS mixing ratio of zero ppt at the corresponding soil moisture. This is based on the linear relationship between OCS uptake and OCS mixing ratio shown by Kesselmeier et al. (1999) and the assumption that the ambient OCS mixing ratio does not influence OCS production (see Section 4.8). This net exchange rate in turn can be determined from the linear regression of the net exchange rates was measured at two atmospheric OCS mixing ratio (50 and 1000 ppt) at the same soil moisture because U_{OCS} at zero ppt atmospheric OCS mixing ratio will be zero. Finally, a CP for a given soil moisture can then be calculated according to Equation (7), where the exchange rate J equals zero $\text{pmol g}^{-1} \text{h}^{-1}$. k_{OCS} , the

consumption rate coefficient, is the slope of the regression between the exchange rate at 50 ppt and 1000 ppt ambient OCS mixing ratio and c is the OCS mixing ratio.

$$J = P_{OCS} + k_{OCS} \times c \quad (7)$$

Deposition velocity V_d [m s^{-1}] was calculated based on the OCS exchange rate $J_{,A}$ [$\text{pmol m}^{-2} \text{s}^{-1}$] and the ambient OCS concentration c [pmol m^{-3}] according to Equation 8.

$$V_d = \frac{J}{c} \quad (8)$$

The calculations of transpiration E [$\text{mmol m}^{-2} \text{s}^{-1}$] and stomatal conductance for water vapor GH_2O [$\text{mmol m}^{-2} \text{s}^{-1}$] for the plant-atmosphere exchange measurements were based on Caemmerer and Farquhar (1981). OCS uptake F_{CO_2} [$\text{pmol m}^{-2} \text{s}^{-1}$] was calculated based on Sandoval-Soto et al. (2005).

Transpiration (E) was calculated according to Equation (9):

$$E = \frac{Q \cdot \Delta H_2O}{LA} \quad (9)$$

where ΔH_2O is the difference in water vapor concentration [mmol mol^{-1}] between sample and reference cuvette, Q [mol s^{-1}] is the flushing rate and LA [m^2] of the enclosed plant.

Stomatal conductance (for water vapor) GH_2O [$\text{mmol m}^{-2} \text{s}^{-1}$] was calculated according to Equation (10):

$$GH_2O = \frac{E}{w_i - w_a} * \left(1 - \frac{w_i + w_a}{2}\right) \quad (10)$$

where w_i is the water vapor concentration [mmol mol^{-1}] in the substomatal space and w_a is the water vapor concentration in the sample cuvette.

The water vapor concentration in the substomatal space w_i can be calculated following the Equation of Goff-Gratch (List, 1984) assuming a water vapor saturation of 100% in the substomatal space.

$$\log_{10}[SVP] = -7.90298 * \left(\frac{T_s}{T} - 1\right) + 5.02808 * \log_{10}\left(\frac{T_s}{T}\right) - 1.3816 * 10^{-7} * \left(10^{11.344 * \left(1 - \frac{T}{T_s}\right)} - 1\right) + 8.1328 * 10^{-3} * \left(10^{-3.49149 * \left(\frac{T_s}{T} - 1\right)} - 1\right) + \log_{10}(SP_{ws}) \quad (11)$$

where SVP is the saturation vapor pressure above a water surface at a given temperature, T is the leaf temperature [K], T_s is (373.16K) and SP_{ws} is the saturation vapor pressure of water at the boiling point.

OCS-uptake F_{COS} [$\mu\text{mol m}^{-2} \text{s}^{-1}$] was calculated according to Equation (12):

$$F_{COS} = \frac{Q * \Delta COS}{LA} - E * COS_{out} \quad (12)$$

Where $COS_{out} = (K1COS + \Delta COS)$, $K1COS$ is the OCS concentration in the reference cuvette and ΔCOS is the concentration difference between sample cuvette and reference cuvette.

Leaf area was determined by copying the contours of all leaves enclosed during a measurement to paper and subsequently scanning the contours with a commercial scanner (EPSON PERFEKTION 3170 Photo, Epson). Contours were filled and their area then determined with the „Compu Eye, Leaf & Symptom Area “software. This software was provided by Dr. Ehab Bakr (Bakr 2005). Deviation of measured areas from dummy leaves with known surface was less than 2%.

3 Results

3.1 Soil-atmosphere exchange at varying soil moisture and at different CO₂ mixing ratios

Figure 7 demonstrates in detail the exchange behavior of the soils as a function of the soil water content. For the untreated (“natural, non-sterilized”, see experimental procedure) soils investigated, the exchange behavior of the four arable soils (see Section 2.2.1) in the first group of experiments, depended strongly on soil moisture. They exhibited a pattern of three distinguishable exchange rate ranges related to soil moisture, consisting of one range with an OCS uptake (or low OCS emission) and two ranges with an OCS emission. An exchange under dry conditions (LSM, low soil moisture) always tended to an emission of OCS. At optimum soils moisture (OSM) an uptake was preferred and at high soil moisture (HSM) a clear tendency towards emission was observed. Increasing CO₂ levels led to a strong OCS emission, except for the Nördlingen Sugar Beet soil.

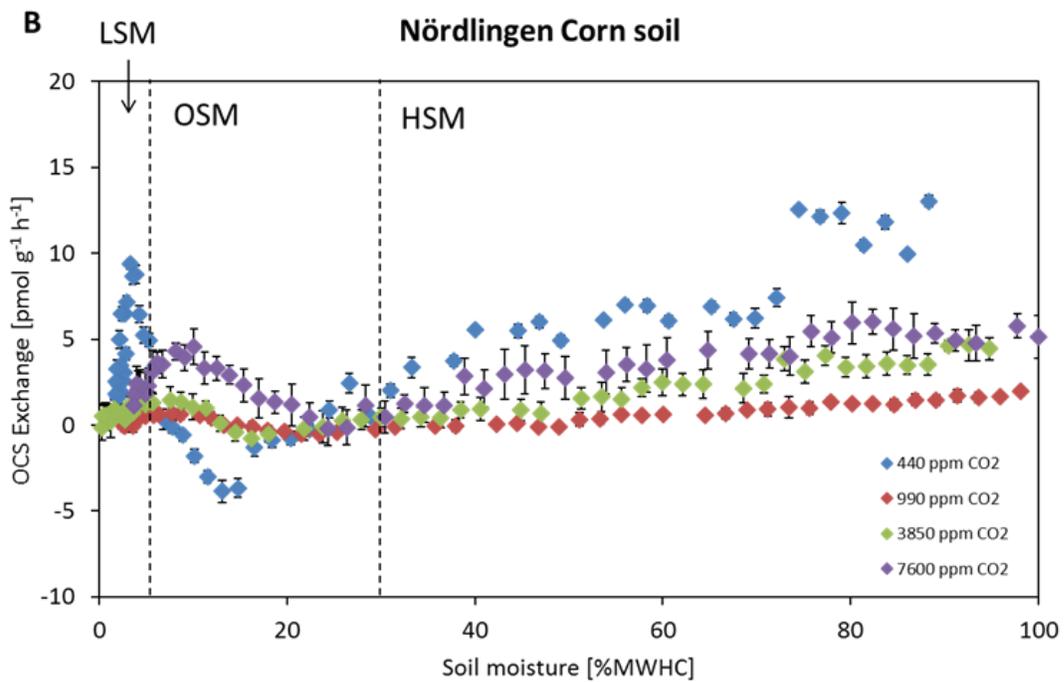
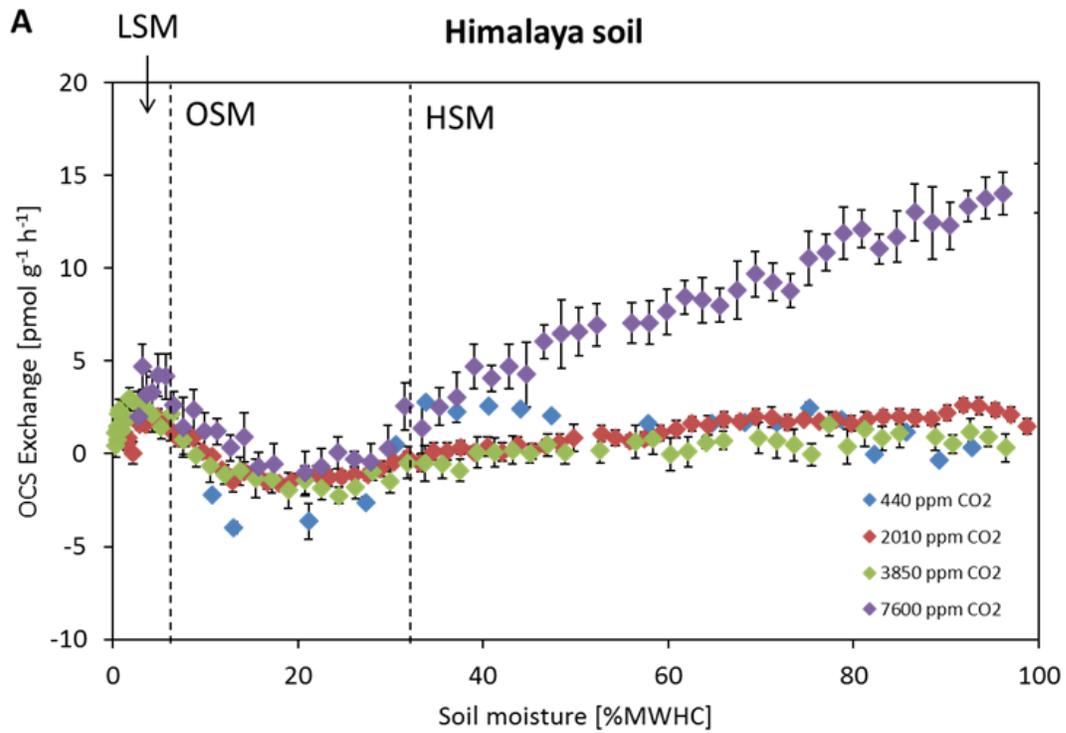


Figure 7 A, B: see next page for captions

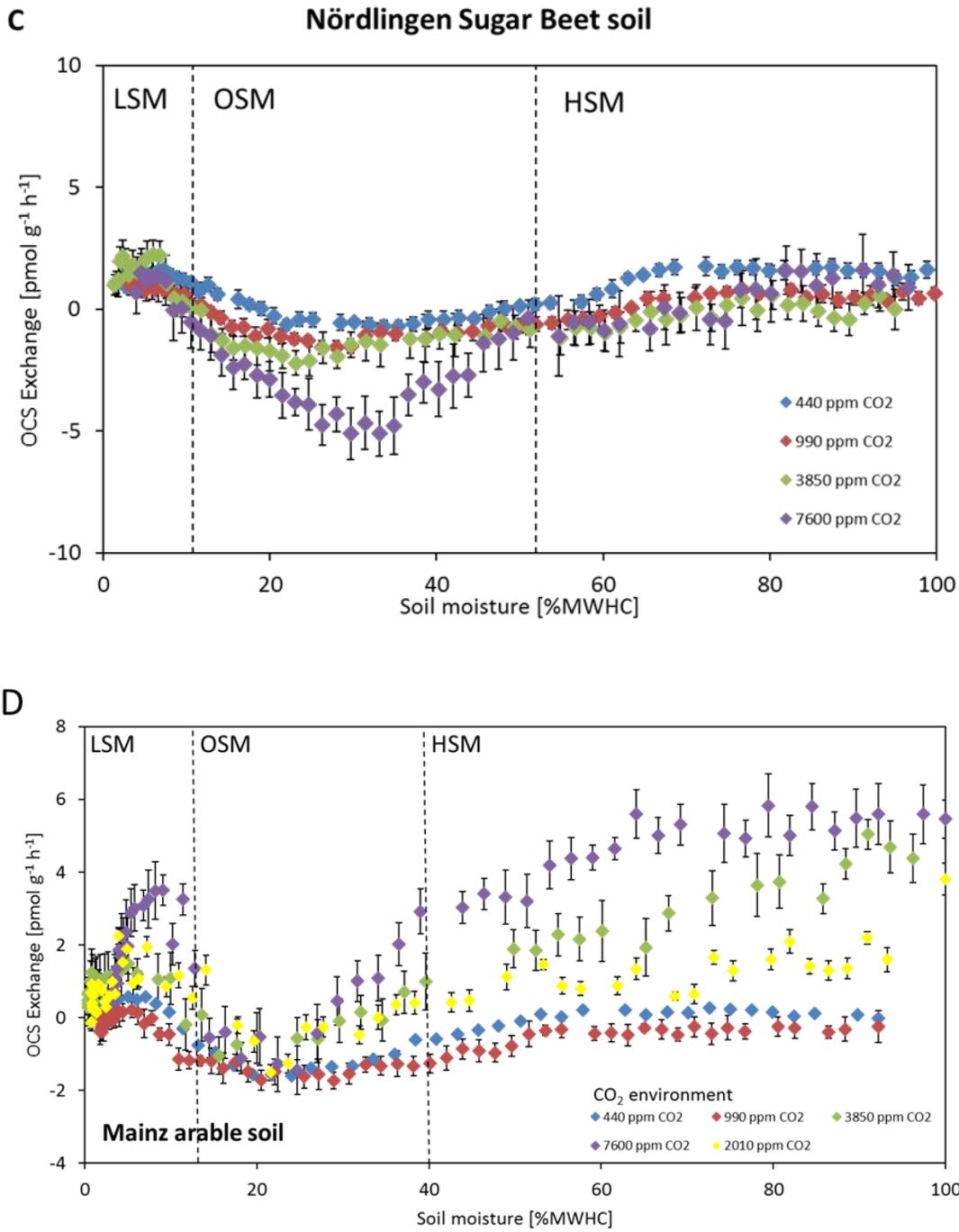


Figure 7 OCS exchange rates for the Himalaya (A), Nördlingen Corn (B), Nördlingen Sugar Beet (C) and Mainz arable (D) soils related to dry weight in $\text{pmol g}^{-1} \text{h}^{-1}$ as a function of the soil moisture given as % MWHC. Negative exchange rates indicate OCS uptake. Dotted lines indicate the areas defined for the integration. LSM, OSM, HSM = low, optimum and high moisture range. Some error bars are smaller than individual points. For experimental conditions see Table 4.

For a better overview, the exchange rates were integrated over the defined ranges of moisture. These ranges of soil moisture are summarized in Table 6, the borders between ranges marked in Figure 7. The integrated rates are shown in Figure 8. Within the low soil moisture range, OCS emission of the “Mainz”, “Himalaya” and “Nördlingen corn” soils increased with rising CO₂ concentration. The “Nördlingen sugar beet” soil also showed OCS emission in this soil moisture range, but no change with increasing CO₂ concentration. Under optimal soil moisture conditions, a more general uptake behavior could be found. The trend of OCS exchange at optimal soil moisture was an uptake of OCS that was reduced and finally changed to an emission with rising ambient CO₂ mixing ratio for “Mainz”, “Himalaya” and “Nördlingen corn” soil. In contrast, the trend for “Nördlingen sugar beet” was an increase of OCS uptake at optimal soil moisture with increasing CO₂ mixing ratio. The Himalaya soil exhibited an increase of the uptake with increasing CO₂ only up to 3850 ppm, followed by an emission under 7600 ppm CO₂. At high soil moisture, an emission was found almost exclusively for all soils with a strong increase at increasing CO₂ levels, except for the “Nördlingen sugar beet” soil. It is noteworthy to point out that the exchange rates (emission rates) at high soil moisture ranged an order of magnitude higher than at lower soil moistures.

Table 6: Definition of soil water content ranges where emission and uptake were observed (in percent of maximum water holding capacity [% MWHC])

	High soil moisture (emission range) [% MWHC]	Low soil moisture (emission range) [% MWHC]	Optimum soil moisture (uptake range) [% MWHC]
Mainz	41-100	3-10	12-40
Nördlingen corn	32-100	2-6	7-31
Nördlingen sugar beet	58-100	3-10	17-57
Himalaya	36-100	0.5-8	9-35

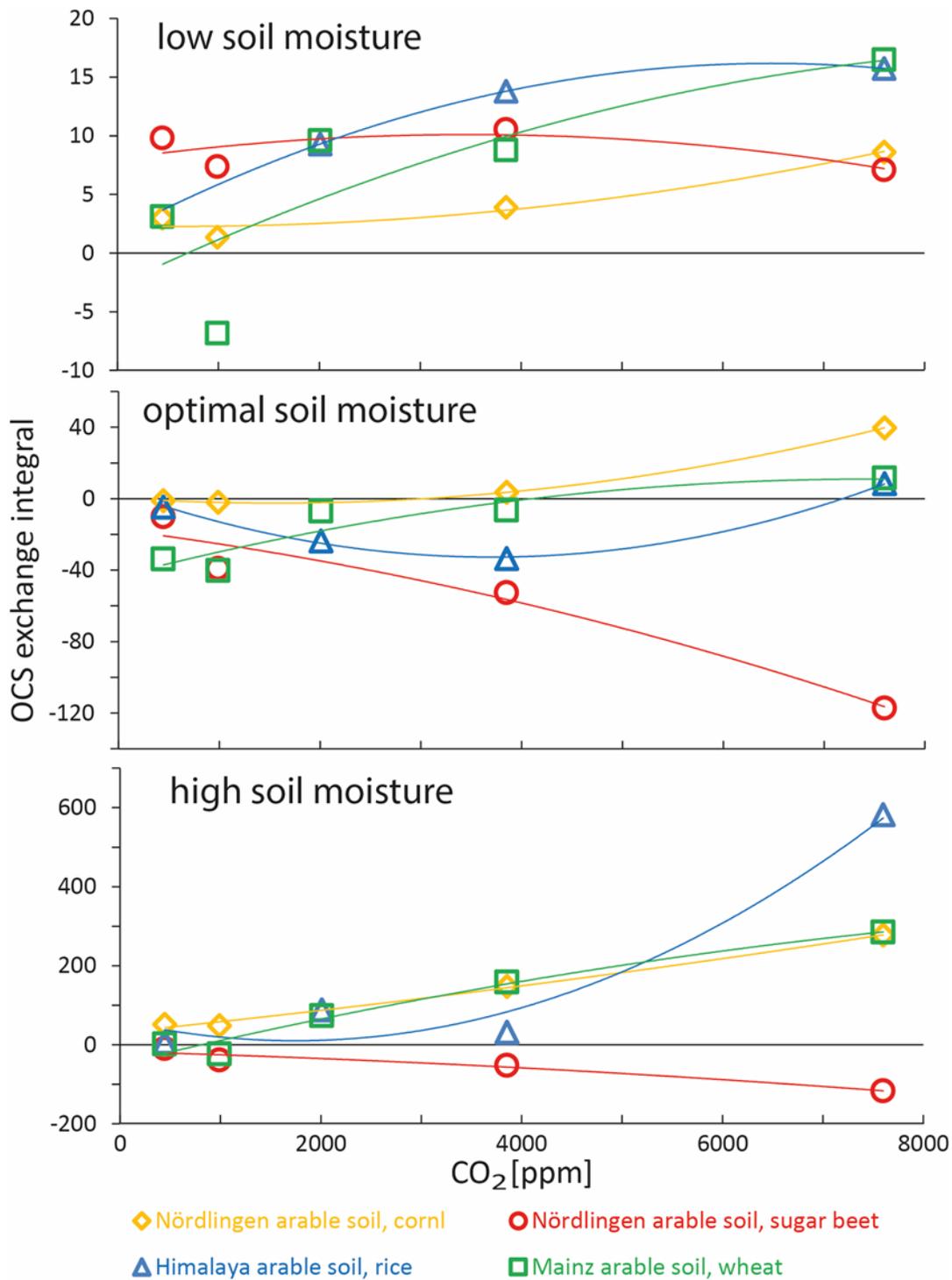


Figure 8 OCS exchange patterns for the four arable soils “Mainz”, “Himalaya”, “Nördlingen corn” and “Nördlingen sugar beet” at CO₂ mixing ratios from 442 to 7601 ppm. Exchange rates have been integrated for high, optimal and low soil moisture. Negative values denote uptake, positive values emission of OCS. For experimental conditions see Table 4.

3.2 Soil-atmosphere exchange after chloroform vapor treatment

In view of the exchange patterns as described in the previous chapters, it was of interest to get a first impression of the microbiota involved. Fumigation by chloroform significantly affected the OCS exchange at optimal soil moisture (Figure 9). OCS uptake by the “Mainz” soil increased strongly under higher CO₂ concentrations while at low concentrations uptake was reduced. It did not switch over to any emission. OCS exchange with “Nördlingen corn” soil switched over to an emission, dropped to an uptake at 4030 ppm and then exhibited emission again at 7520 ppm, though only half of the amount as found without sterilization (see Figure 8). For “Nördlingen sugar beet” soil, which showed increasing uptake with increasing CO₂ only when untreated, an increase of the uptake at lower CO₂ and a reduction of the uptake at 7520 ppm were observed.

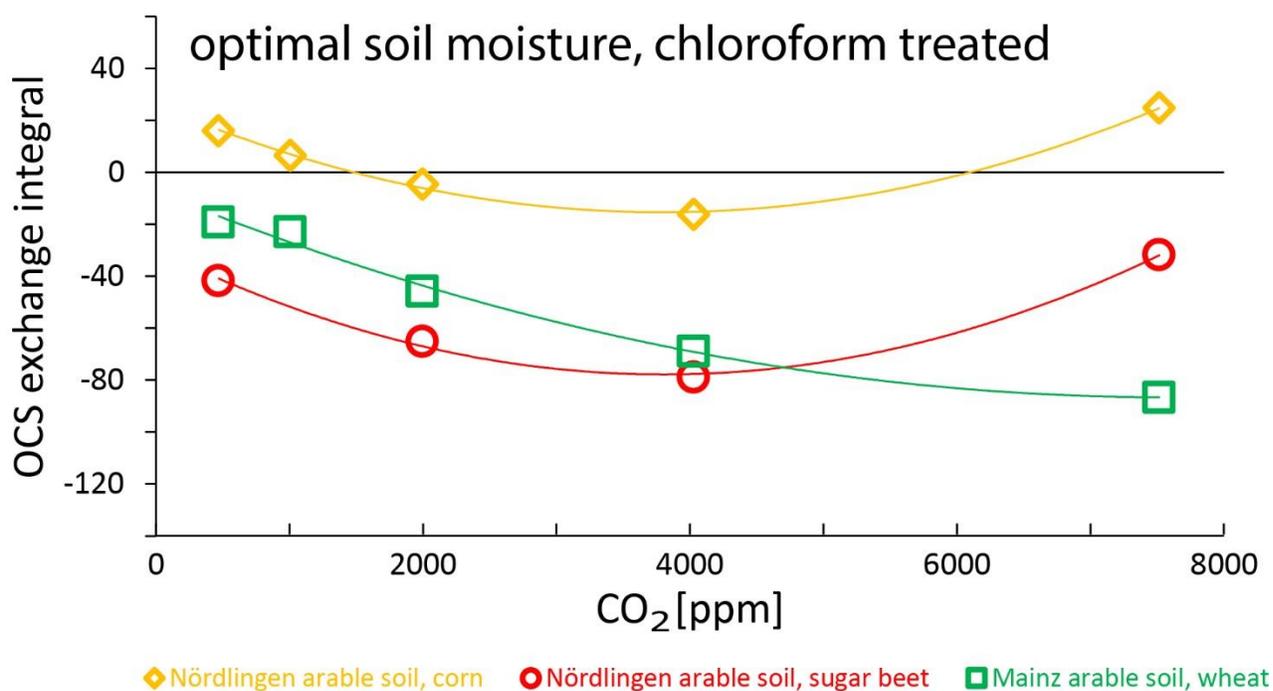


Figure 9: OCS exchange at different CO₂ concentrations after fumigation with chloroform vapor. Negative values denote uptake, positive values emission of OCS. All measurements were performed at optimal soil moisture. For experimental conditions see Table 4.

3.3 Soil-atmosphere exchange after nystatin or streptomycin treatment

While chloroform acts more generally, treatment with antifungal and antibiotic compounds may allow a more specific interpretation. Therefore, the agents nystatin (known as inhibitor of fungi) and streptomycin (known as inhibitor of bacteria, see Section 2.4) were applied. Only small differences between untreated and streptomycin treated samples were found. However, a significant shift towards emission was observed with nystatin (Figure 10) over the whole range of soil moisture conditions for the “Mainz” soil sample. The “Suriname” soil sample, exhibiting almost no variability related to soil moisture, showed a higher emission under nystatin and streptomycin treatment with strongest effects under nystatin.

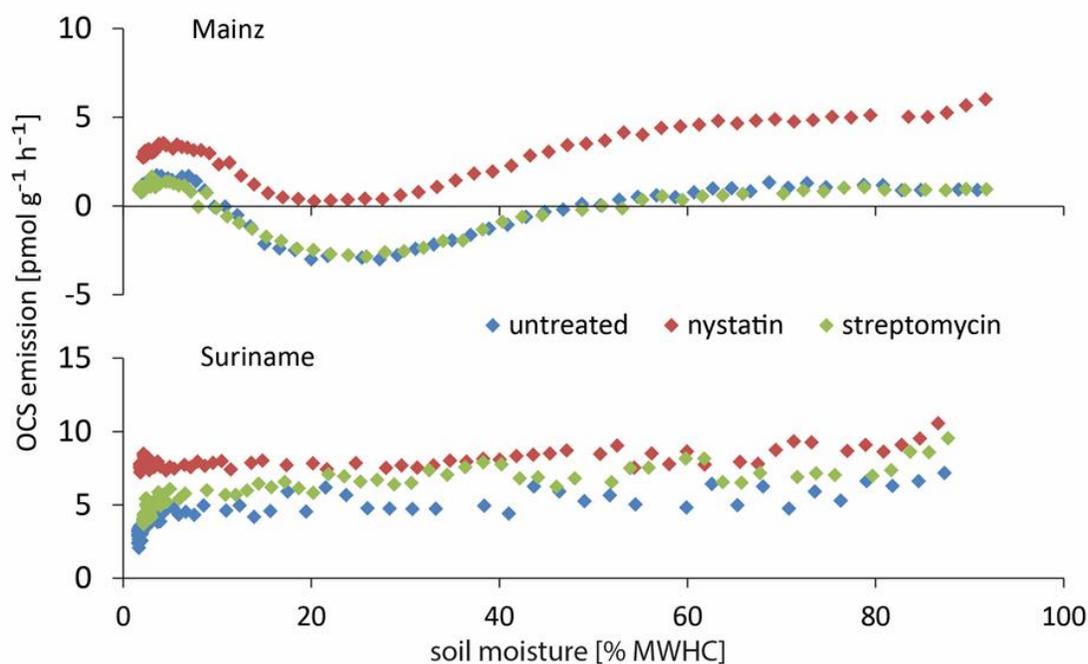


Figure 10 OCS exchange rate of untreated (blue diamonds), nystatin treated (red diamonds), and streptomycin treated (green diamonds) soil samples at 925 ppt OCS mixing ratio and 430 ppm CO₂ mixing ratio. Error bars are smaller than individual points.

3.4 OCS exchange for organic layers and litter layer from needle forests in comparison to an arable soil

The exchange of OCS between the needle forest soils and the atmosphere was investigated at 50 and 1000 ppt ambient OCS and compared with the exchange pattern of the Mainz soil (Figure 11). All four soils showed OCS uptake at medium soil moisture when ambient OCS was high (1000 ppt). The uptake was reduced or switched to emission at high and low soil moisture. The two Waldstein soils had a rather broad uptake peak with its maximum at around 40% MWHC. OCS uptake at medium soil moisture was 20% stronger for the Waldstein soil with a young spruce understory than for the one with a blueberry understory. Furthermore, the slope between maximal uptake at medium soil moisture and lower uptake at low or high soil moisture was steeper with young spruce understory.

In comparison to the Waldstein soils, the uptake peaks of the Finland and the Mainz soils were sharper and located around 18 % and 20 % MWHC, respectively, at lower soil moisture. At higher soil humidity the Mainz soil emitted OCS while the emission was very low for the Finland soil. The maximal net uptake varied between soils, ranging from 3 pmol g⁻¹ h⁻¹ (Mainz soil) over 13 pmol g⁻¹ h⁻¹ (Waldstein Blueberry) and 23 pmol g⁻¹ h⁻¹ (Waldstein Spruce) to 85 pmol g⁻¹ h⁻¹ (Finland Needle Forest) as shown in Figure 11. The corresponding uptake rates are 5, 25, 32 and 110 pmol g⁻¹ h⁻¹ respectively.

At low ambient OCS mixing ratio (50 ppt), all soils showed OCS emission that was mostly constant at any soil moisture, except some decline at very low soil humidity. Emission strength varied between soils, ranging from about 1 pmol g⁻¹ h⁻¹ (Waldstein Blueberry) over 2 pmol g⁻¹ h⁻¹ (Mainz Soil) and 3 pmol g⁻¹ h⁻¹ (Waldstein Spruce) up to 15 pmol g⁻¹ h⁻¹ (Finland Needle Forest).

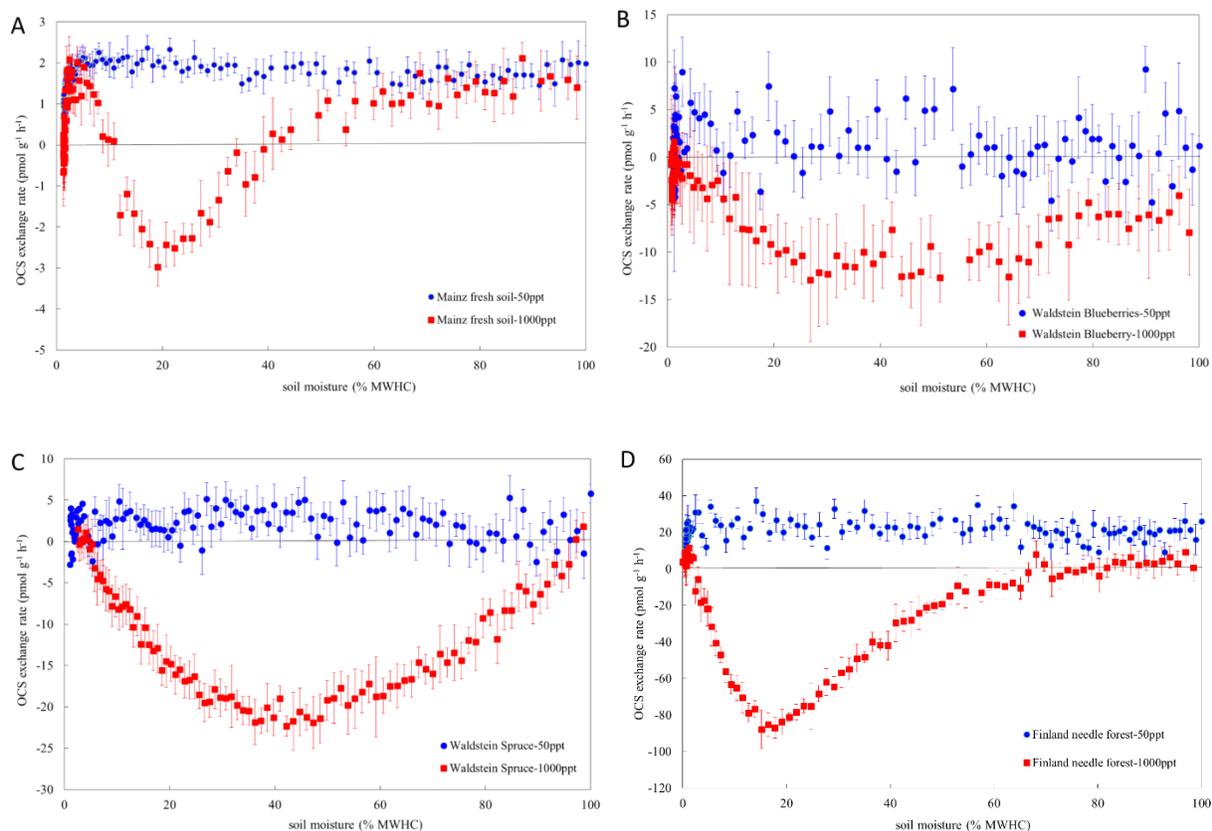


Figure 11 COS net exchange rates for different fresh soil at 50 and 1000 ppt ambient COS in relation to soil moisture in % MWHC (A: Mainz arable soil; B: Waldstein Blueberry soil; C: Waldstein Spruce soil; D: Litter layer Finland needle forest soil). For experimental conditions see Table 5.

For the Mainz soil, both after storage air dried or fresh, the influence of the OCS mixing ratio was examined in more detail by measuring OCS exchange at an additional mixing ratio of 500 ppt. As before, OCS exchange strongly correlated with ambient OCS mixing ratio and soil humidity. Net exchange is shown in Figure 12. For fresh Mainz soil, at high OCS mixing ratio (1000 ppt), the exchange behavior followed the basic pattern of emission-uptake-emission (from wet to dry soil), as already observed by Bunk et al. (2017). At 500 ppt ambient OCS mixing ratio, the uptake in the medium humidity range was reduced, in comparison to the uptake at 1000 ppm OCS mixing ratio. Emission at high and low humidity was similar to the 1000 ppt experiment. At 50 ppt ambient OCS, there was a nearly constant emission of OCS of about $2 \text{ pmol g}^{-1} \text{ h}^{-1}$. For the Mainz soil that was air dried before storage, the exchange patterns were similar, but a general decrease of the

OCS emission rates and increase in uptake rates was found. OCS release at low and high humidity as well as under low ambient OCS mixing ratios were lower. Uptake in the medium humidity range was stronger, especially at high ambient OCS. Under high ambient OCS mixing ratio (1000 ppt), the OCS exchange at low humidity did not any reach emission. Additionally, the uptake peak at medium humidity and high ambient OCS was broader.

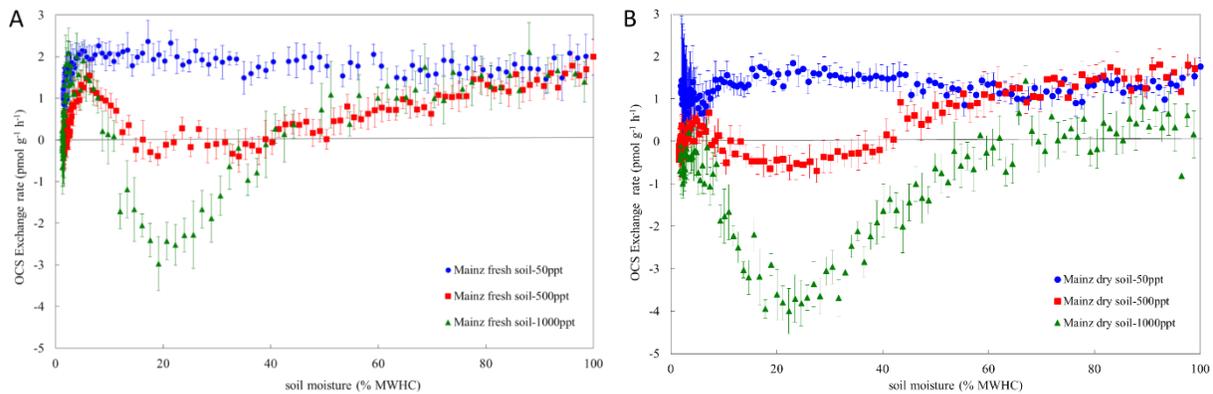


Figure 12 COS net exchange rates in relation to soil moisture at 20° C for 80 g of fresh and dry arable soil per cuvette. Different ambient COS mixing ratios with ~50 ppt (blue circle), ~500 ppt (red square), or ~1000 ppt (green triangle) OCS in the flushing air were applied. (A: fresh soil; B: dry soil). For experimental conditions see Table 5.

3.5 OCS compensation points

OCS compensation points were found to be variable in close dependence to the soil water content. The Mainz (dry and wet storage) soil, Finland litter layer, and Waldstein Spruce soil showed high compensation points for wet and dry soil and lower compensation points at a range of humidity between the wet and dry extremes (see Figure 13). CPs were in the range of 300 to 5500 ppt, 130 to 320 ppt, 180 to 1150 ppt and 210 to 1730 ppt for Mainz soil, Waldstein Blueberry soil, Waldstein Spruce soil and litter layer Finland soil, respectively. The Mainz soil and litter layer Finland soil had their lowest CP in the moisture range of roughly 15 and 40 % MWHC. Waldstein blueberry soil had its highest CP in the extremely dry and in the moisture range of 73 to 80%

MWHC, while the other 3 soils had their highest CP in the extremely dry and extremely wet. These data were compared to reports by Lehmann and Conrad 1996 (CP for: PBE, a forest soil without small vegetation (Marburg); BW, a forest soil vegetated with *Dryopteris assimilis*, *Mnium undulatum* and *Leucobryum glaucum* (Schachtenau) and BL, a forest soil vegetated with *Allium ursinum* (Radolfzell). The soil samples PBE, BW, and BL were reported to be measured at approximately 43%, 44% and 58% MWHC, respectively. The range for the CP as extracted from Kesselmeier et al. (1999) is reflecting the fluctuation of a sample from the same area as investigated by the current work, but under different temperatures.

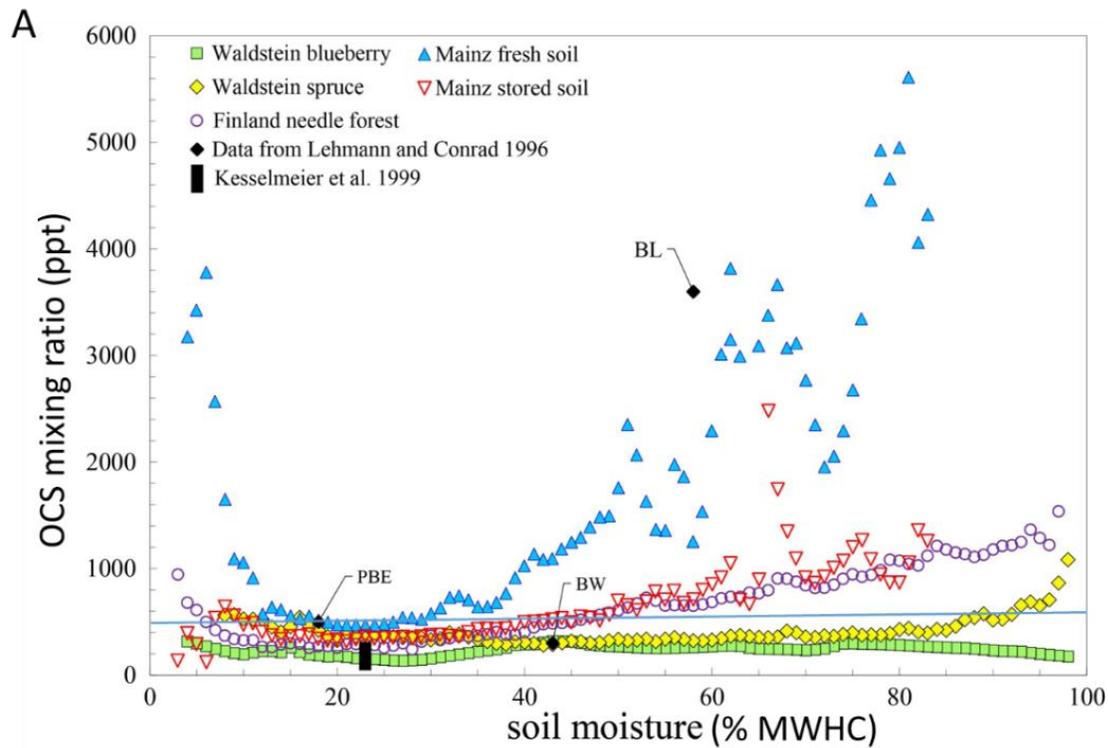


Figure 13 OCS compensation mixing ratios (CP, when $E_{ocs}=0$) at different soil water contents of four soils. CP were calculated based on the net exchange rate under the ambient OCS mixing ratio of ~ 1000 ppt and ~ 50 ppt, based on Equations (6) and (7) (see Section 2.6). For comparison compensation points calculated by Lehmann and Conrad 1996 (see Sections 3.5 and 4.8) were added as black diamonds (PBE, BW and BL, see Section 3.5), compensation points from Kesselmeier et al (1999) (see Sections 3.5 and 4.8) were added as a black bar. The bar shown represents the range of compensation points, with its lower edge representing the lowest and its upper edge representing the highest CP. The continuous blue line marks the average tropospheric OCS mixing ratio of the northern hemisphere.

3.6 Production (P_{OCS}) and consumption (U_{OCS}) in relation to soil moisture

P_{OCS} did not vary significantly with soil moisture, except for the Waldstein soil with blueberry understory. For the Waldstein blueberry soil, P_{OCS} increased slightly at moderate soil moisture. Average P_{OCS} was 2, 4, 7 and 30 $\text{pmol g}^{-1} \text{h}^{-1}$ for Mainz soil, Waldstein soil with blueberry or young spruce understory and Finland needle forest litter, respectively. U_{OCS} on the other hand was strongly influenced by soil moisture, with a maximum at medium soil moistures and lower U_{OCS} at low and high soil moisture. The maximum/minimum OCS was 5/0, 25/5, 33/8 and 120/20 $\text{pmol g}^{-1} \text{h}^{-1}$ for Mainz, Waldstein Blueberry, Waldstein Spruce soil and Finland litter layer, respectively. U_{OCS} , P_{OCS} and the corresponding exchange rates are shown in Figure 14.

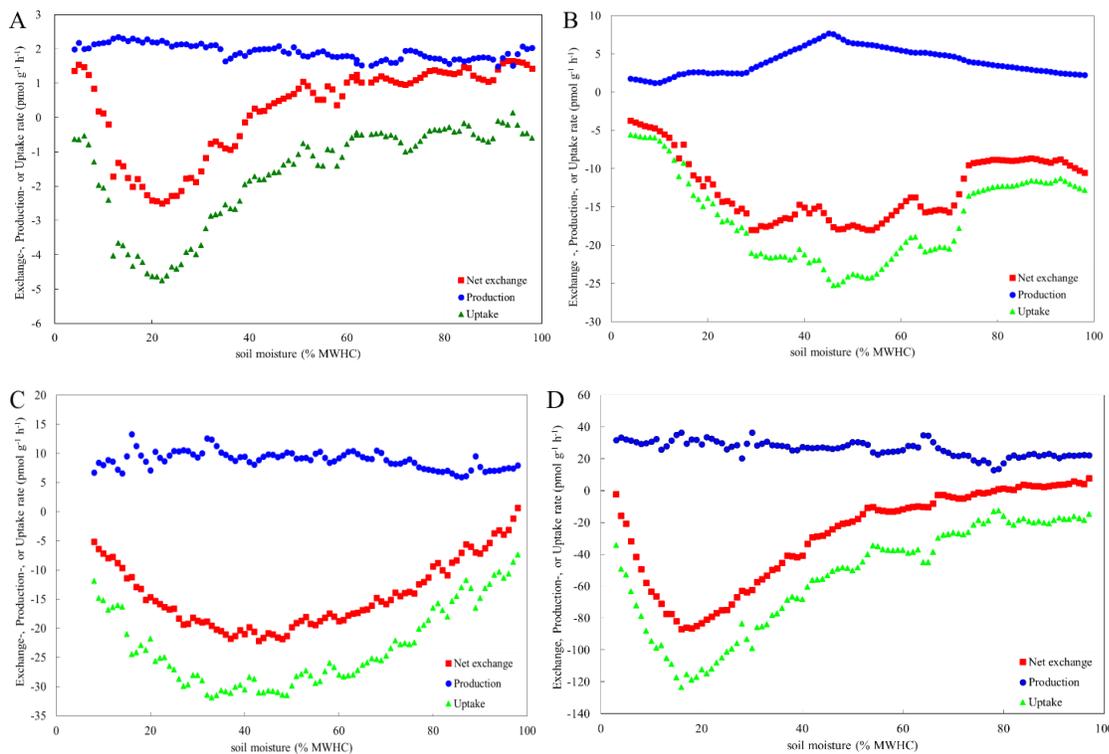


Figure 14 Uptake rate (U_{OCS}) and production rate (P_{OCS}) calculated as described in Section 2.6 and net exchange rate (J) measured at 1000 ppt OCS mixing ratio for A: Mainz soil, B: Waldstein spruce soil (blueberry understory), C: Waldstein spruce forest soil (young spruce understory) and D: Finland needle leaf forest.

3.7 Outlook: plant-atmosphere exchange at different CO₂ mixing ratios

This chapter gives an overview about measurements, which are complementary to earlier investigations on the effect of elevated CO₂ (White et al., 2010; Sandoval-Soto et al., 2012). In contrast to these earlier works, we increased the CO₂ concentrations to high levels, corresponding to those used for soil experiments. Figures 15 to 21 show the OCS uptake and stomatal conductance of the tested plants at different CO₂ mixing ratios.

Quercus ilex and *Fagus sylvatica* OCS trace gas exchange was measured at a range of lower CO₂ mixing ratios in spring 2015 (up to 2000 ppm) and at a range of high CO₂ mixing ratios (up to 4900 or 7500 ppm respectively) in summer 2016 using a different individual for either species. Both species show lower OCS uptake and stomatal conductance in the 2016 measurements.

At CO₂ mixing ratios lower than 5000 ppm, OCS uptake (F_{CO_2}) declined with increasing CO₂ mixing ratio and was very closely correlated with stomatal conductance ($G_{\text{H}_2\text{O}}$) for all plants examined. For *Zea mays* the correlation between stomatal conductance and OCS uptake persisted at all CO₂ mixing ratios. The sole exception was *Fagus sylvatica* tree no. 15. For this individual, OCS uptake slightly increased up to a CO₂ mixing ratio of 1650 ppm and then declined. This uptake behavior still was very closely correlated to the stomatal conductance. At higher CO₂ mixing ratios, 4000 ppm for *Fagus sylvatica* (tree no. 9) and 4500 ppm for *Quercus robur*, the close correlation between OCS uptake and stomatal conductance started to break down for *Fagus sylvatica*, *Sorbus aria* and *Quercus robur*. OCS uptake increased again, while the stomatal conductance declined further (*Sorbus aria* and *Fagus sylvatica*) or proportionally increased to a much lesser degree (*Quercus robur*).

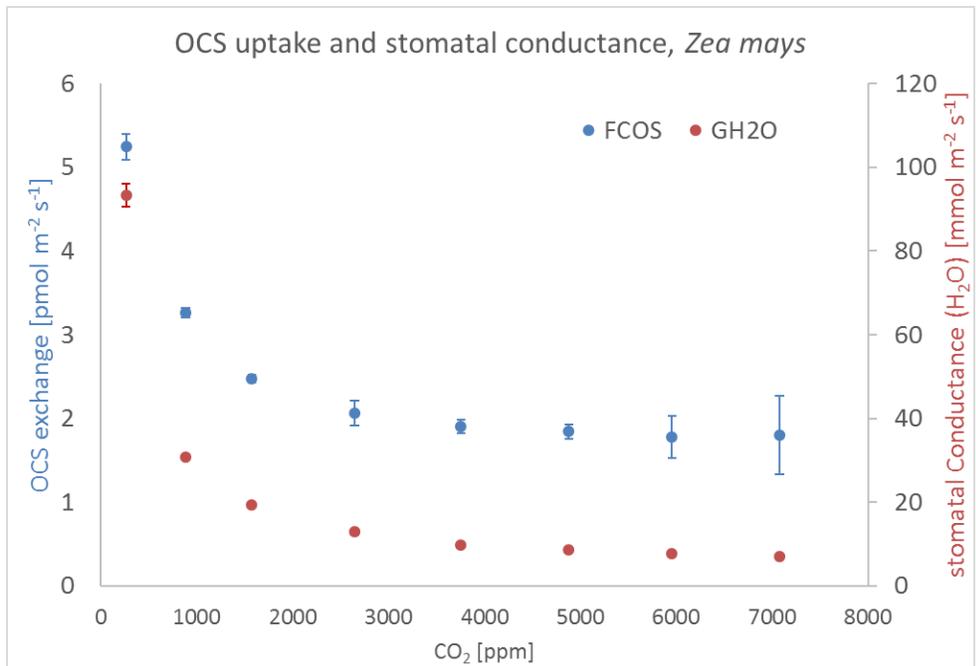


Figure 15 OCS exchange (positive values indicate OCS uptake) and stomatal of *Zea mays* at different CO₂ regimen. Error bars denote the standard deviation. See Section 2.5 for experimental conditions.

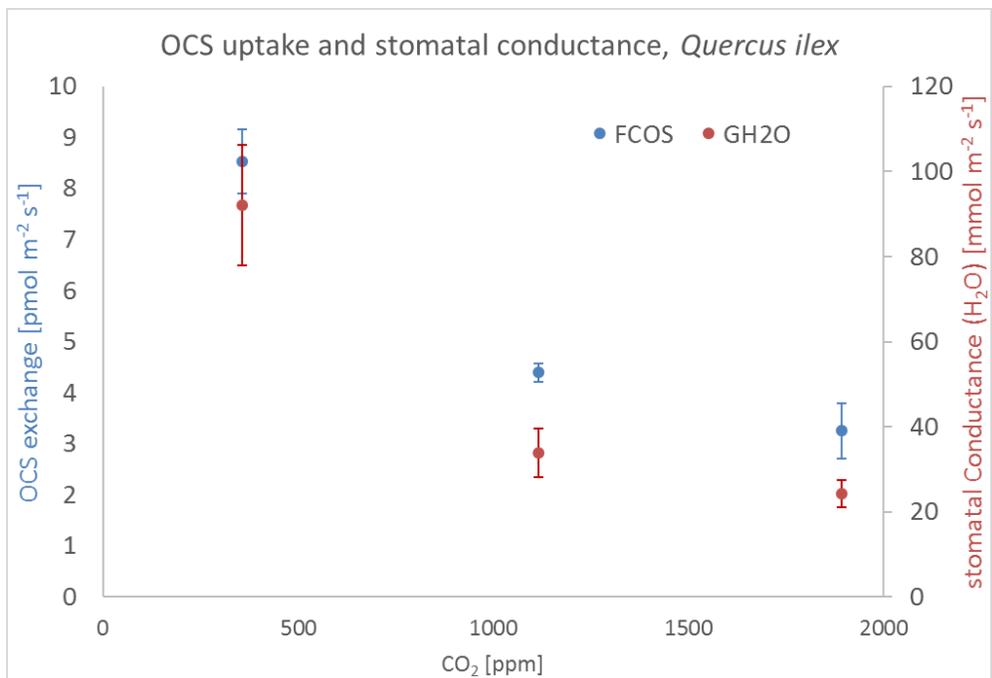


Figure 16 OCS exchange (positive values indicate OCS uptake) and stomatal of *Quercus ilex* (tree no. 4) at different CO₂ regimen. Error bars denote the standard deviation. See Section 2.5 for experimental conditions.

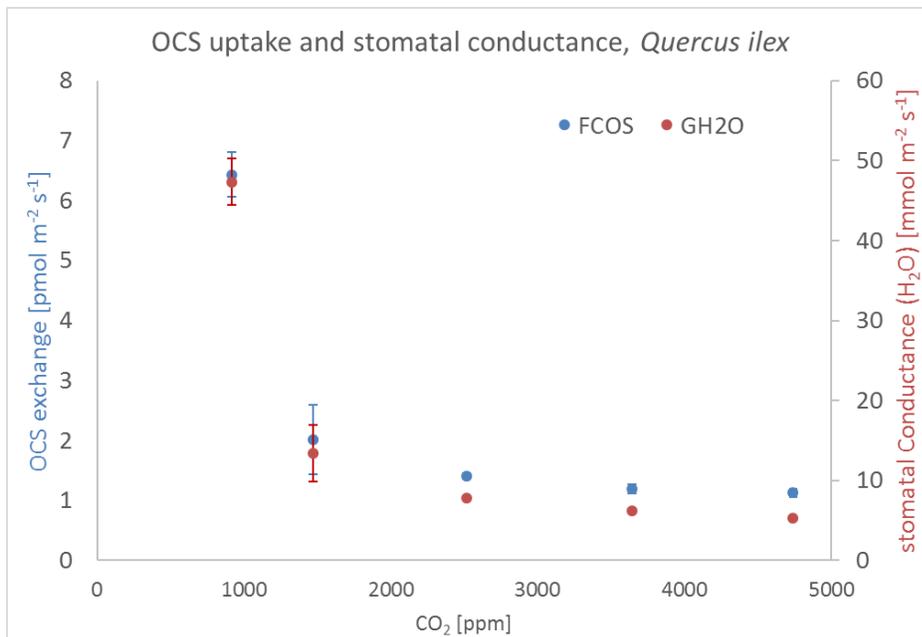


Figure 17 OCS exchange (positive values indicate OCS uptake) and stomatal of *Quercus ilex* (tree no. 2) at different CO₂ regimen. Error bars denote the standard deviation. See Section 2.5 for experimental conditions.

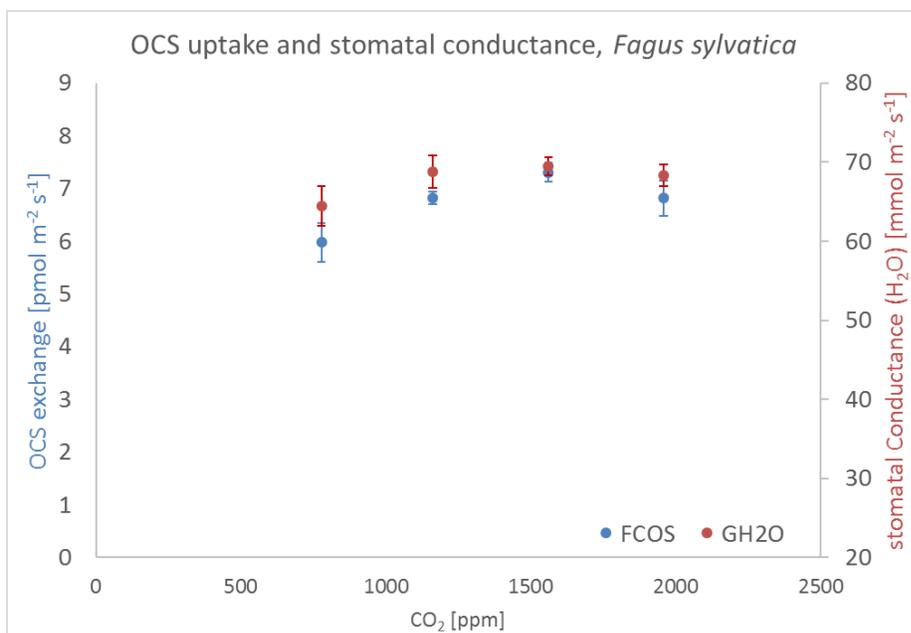


Figure 18: OCS exchange (positive values indicate OCS uptake) and stomatal of *Fagus sylvatica* (tree no. 15) at different CO₂ regimen. Error bars denote the standard deviation. See Section 2.5 for experimental conditions.

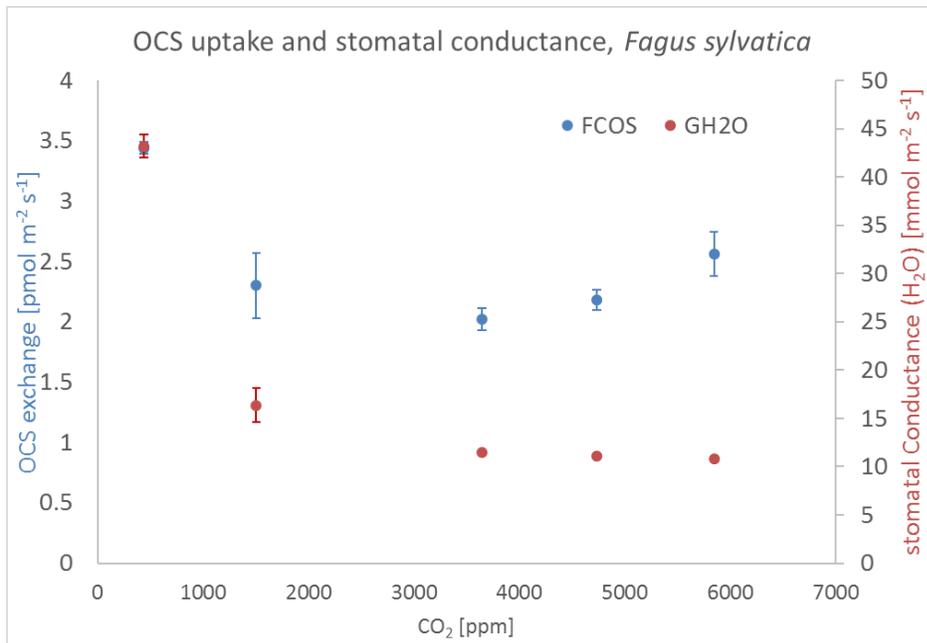


Figure 19 OCS exchange (positive values indicate OCS uptake) and stomatal of *Fagus sylvatica* (tree no. 9) at different CO₂ regimen. Error bars denote the standard deviation. See Section 2.5 for experimental conditions.

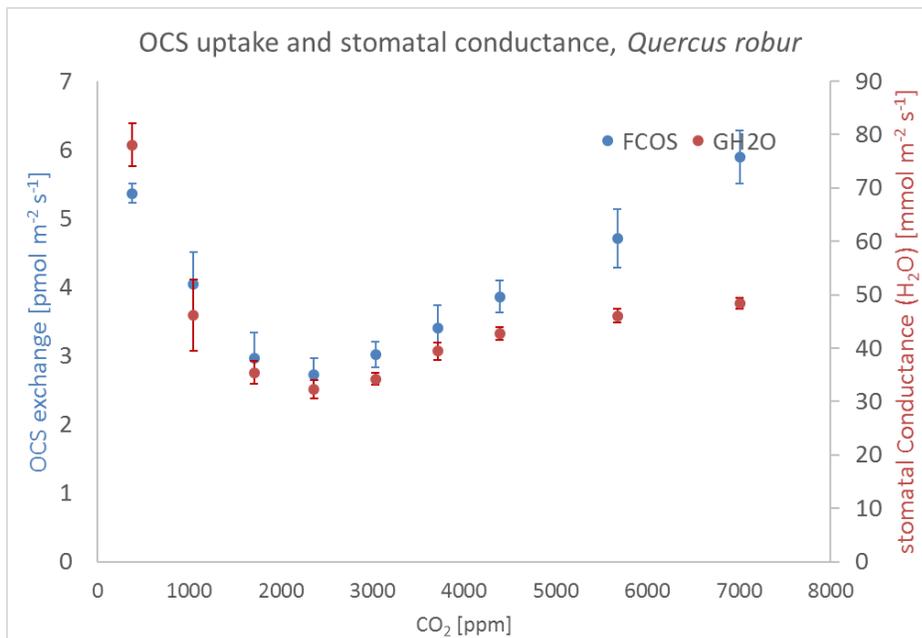


Figure 20 OCS exchange (positive values indicate OCS uptake) and stomatal of *Quercus robur* at different CO₂ regimen. Error bars denote the standard deviation. See Section 2.5 for experimental conditions.

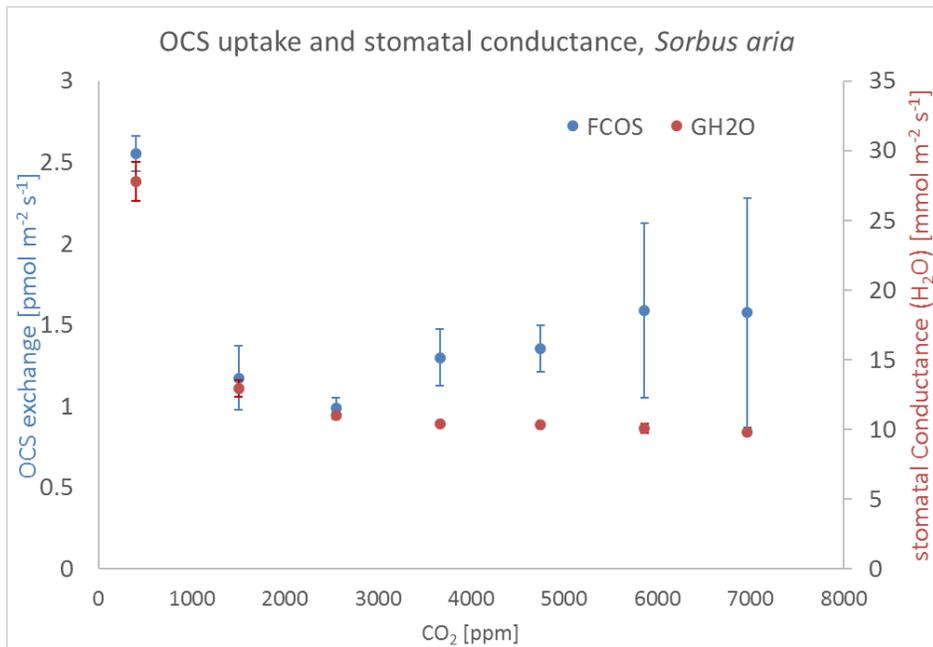


Figure 21 OCS exchange (positive values indicate OCS uptake) and stomatal of *Sorbus aria* at different CO₂ regimen. Error bars denote the standard deviation. See Section 2.5 for experimental conditions.

3.8 Outlook: OCS uptake and water vapor release in the light and dark before and after Fusicoccin treatment

Effects of high levels of CO₂, as detected with soils, could not be directly demonstrated with plants due to the effect of stomatal closure. Therefore, fusicoccin, a wilting toxin, which triggers stomata to stay open, was applied. The effect is reported in figure 22, showing the OCS concentrations in the plant and reference cuvette under different light conditons.

Prior to the application of Fusicoccin the measured OCS mixing ratio in the light changed with every switch from plant to refrenec cuvette every ten minutes between 520 ppt and 465 ppt on average (Figure 22, top and bottom left). This change always occurred when the Analyzer inlet was switched from the sample cuvette (containing the *Quercus robur* branch) and the empty reference cuvette. The difference in mixing ratio thus represents the uptake by the branch. The difference in water vapor concentration during that time was 8 mmol mol⁻¹ on average (Figure

22, bottom left), representing the water vapor release by the branch. When the light was turned off, the difference in OCS mixing ratio was reduced to less than 7 ppt on average being barely visible in Figure 22. The difference in water vapor concentration was reduced by 6 mmol mol⁻¹ to 2 mmol mol⁻¹ on average.

After the addition of Fusicoccin the difference in OCS mixing ratio between sample and reference cuvette stayed constant at about 20 ppt (top and bottom right) when the light was turned off. The difference in water vapor concentration was reduced by 1 mmol mol⁻¹ (From 6 mmol mol⁻¹ to 5 mmol mol⁻¹; bottom right).

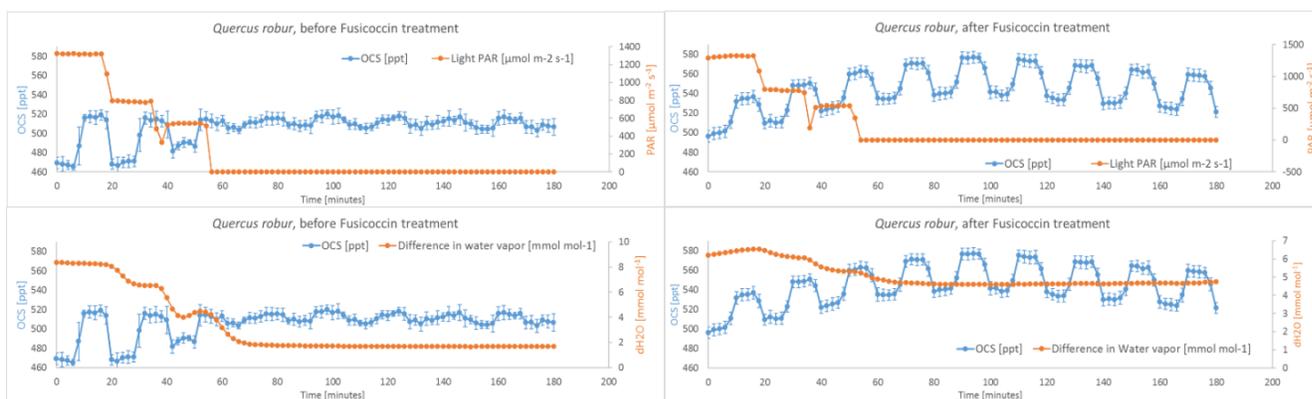


Figure 22: OCS mixing ratio and light intensity before (top left) and after (top right) Fusicoccin application and differential of water vapor concentration plus OCS mixing ratio between sample and reference chamber and OCS mixing ratio before (bottom left) and after Fusicoccin application (bottom right). Error bars denote the standard deviation. See Section 2.5 for experimental conditions.

4. Discussion

The exchange of carbonyl sulfide (OCS) between biosphere and the atmosphere is a complex issue, especially for soils, which may play a significant role contributing to an exchange on the ecosystem scale. This is of high importance in order to decide upon the use of the so called “OCS-Proxy” for GPP determination. Biotic uptake and consumption (Kesselmeier et al., 1999; Protoschill-Krebs et al., 1996; Seefeldt et al., 1995; Lorimer and Pierce, 1989), biotic production (Jordan et al., 1997; Jordan et al., 1995; Katayama et al., 1992; Smith and Kelly, 1988), abiotic destruction (Lehmann and Conrad, 1996; Elliot et al., 1989), and abiotic production (Whelan et al., 2016; Whelan and Rew, 2015; Lehmann and Conrad, 1996), have been reported either for mineral soils or for the organic fraction, including enzymes or organisms found in soils. Because the observed signal is a composite of the above mentioned processes, which may differ among soil types and environmental conditions, all these sources and sinks must be considered in any net exchange observed.

4.1 OCS exchange at varying soil moisture

Results show a clear dependence of OCS exchange on soil moisture. OCS was released under high and very low soil moisture, whereas there was uptake of OCS in an intermediate moisture range (see Figures 7 and 8). There are two ways soil moisture can influence OCS exchange: microbial activity, and diffusivity:

(i) microbial activity: At different soil moistures, different microbial communities are active (Drenovsky et al. 2004; Cleveland et al., 2007; Gleeson et al., 2010; Oswald et al., 2013). Depending on their enzymatic inventory, this may favor production or consumption.

(ii) diffusivity: To be taken up by enzyme within a cell, OCS has first to reach those cells by diffusion. As a gas, OCS will reach its reaction site the easier, the lower the soil moisture drops. At high soil moisture much of the soil pore space is filled with water, impeding OCS diffusion. As the soil dries, diffusivity improves, allowing faster uptake. Such a dependence of OCS uptake on water filled pore space has been shown by Kesselmeier et al. (1999) and van Diest and Kesselmeier (2008). But also production and release is triggered by diffusivity, which influences oxygen availability. Smith and Kelly (1988) reported OCS production only under anaerobic conditions.

For all four arable soils there is an OCS exchange pattern with OCS emission at high soil moisture followed by OCS uptake at an optimal soil humidity and finally emission again at very low soil moisture. At high soil moisture, diffusivity is low (see ii), limiting OCS uptake. At the same time, enough water to dissolve precursors, such as thiocyanate, is available, allowing the uptake of this precursor by microorganisms. Additionally, conditions are more anaerobic, which may be favorable to some OCS production pathways (Smith and Kelly, 1988)

When the soil moisture declines, reaching “optimal soil moisture”, diffusivity and consequently OCS uptake improve (see ii). In parallel, conditions become more aerobic, worsening conditions for some OCS production pathways.

In addition, at different soil moistures, different microorganisms may be active (see i), possibly facilitating uptake or emission more strongly.

The final peak of OCS emission at very low soil moisture is harder to explain. Some authors report breakage of cells at very low soil water contents (Ermel 2004), which may lead to release of trace gases. Such a process may be regarded as abiotic, but it cannot be excluded this peak being caused by microorganisms adapted to this environment.

The available data does not allow to quantify the extent to which each of the mechanisms discussed above contributes to the observed net exchanges. There is no reason that would disqualify any of the above discussed mechanisms. Also, most of them have been described in the literature previously. It is reasonable to assume that all play a role to some extent. However, the separation of OCS net exchange into consumption (UOCS) and production (POCS) for the Mainz soil and three forest soils (see Sections 2.4, 3.6 and 4.7) suggests that the influence of soil moisture on the consumption might be stronger than on the production. More studies, especially with different approaches, are highly desirable, and projects with isotopic and genetic techniques are already underway (Behrendt et al., 2017).

4.2 OCS exchange under elevated CO₂

Increasing CO₂ up to normal soil levels as reported in the literature (Hirano et al., 2003; Yonemura et al., 2009; Sakurai et al., 2015) affected the observed OCS exchange patterns significantly. With increasing CO₂ at high soil water content, emission strongly increased under 7600 ppm CO₂. Even at optimal humidity for an uptake, the uptake rates were reduced under these conditions and in some cases strong emission was observed, especially at the two highest CO₂ concentrations. Such a change in exchange behavior may be either due to increased production of OCS (i), decreased uptake (ii), or a combination of both.

(i) Increased CO₂ concentrations may boost the activity of OCS producing bacteria. In culture studies, various authors observed increased growth and activity of microorganisms with increasing CO₂ (Dehority 1971; Repaske and Clayton 1978; Samuelov et al., 1991). While Repaske and Clayton observed saturation at 400 ppm CO₂ for *Escherichia coli*, Dehority (1971) reported that many species and strains of bacteria needed a concentration of 1000 ppm atmospheric CO₂ for optimal growth, a few strains even required a 10 % CO₂ atmosphere to reach maximal growth.

In addition to an influence on the growth of *Anaerobiospirillum succiniciproducens*, Samuelov et al. (1991), found that increased levels of $[\text{CO}_2/\text{HCO}_3^-]$ also induced to a shift between metabolic pathways with expression of different enzymes and accumulation of a different end product. Such a shift in the metabolic pathways could be the cause for the strong OCS emission increase at 7600 ppm CO_2 observed for some of the examined soils.

(ii) Enzymatic uptake of OCS may be competitively inhibited. Given the large concentration difference between OCS and CO_2 ($1:10^6$), CO_2 would have to occupy the binding site of all, or nearly all enzyme molecules. An indicator for the substrate concentration at which this state is reached is the K_M value of an enzyme, which describes the substrate concentration at which half the maximal rate of turnover of an enzyme is attained. Beyond this concentration, the enzyme starts getting saturated, until more substrate will not increase conversion speed as all binding sites are occupied. Therefore, the CO_2 concentration in the soil water would have to exceed the K_M value of the enzyme in question. The K_M value of CA (and other enzymes) is not uniform throughout species and also depends on pH and temperature. The BRENDA database (Schomburg et al., 2002; www.brenda-enzymes.org) offers K_M values for CA and CO_2 as substrate that, excluding macro-fauna, macro-flora and marine algae, range from 0.089 to 80 mM. The reported values cluster in the range of 1-20 mM, with median value of about 17 mM (when averaging different entries for the same species). This fits rather well with the K_M values reported for bacterial CA by Chirică et al. (1997) (20 mM, CA typ A) and Nishimori et al. (2007) (14.7 mM, CA typ B). Only one entry in BRENDA is below 0.3 mM (or 5 out of 256 entries if none are omitted). Ogée et al. (2016) suggest a K_M value of about 3 mM as typical for CA in soils.

The equilibrium of CO_2 (or any other gas) between the gas phase and the liquid phase is described by Henry's law. Henry's law takes many forms, one of them is $H^{\text{cp}} = c_a/p$ (Sander 2015). Here c_a is the CO_2 concentration in the aqueous phase in mol m^{-3} , p is the partial pressure of CO_2 in Pa,

and H^{cp} is the Henry Solubility in $\text{mol m}^{-3} \text{Pa}^{-1}$. Sander (2015) lists H^{cp} for CO_2 at 25°C and CO_2 as 3.3×10^{-4} . Corrected for 20°C by eq. 19 in Sander 2015 H^{cp} (20°C) is 3.78×10^{-4} .

Based on the Henry equilibrium and the CO_2 concentration in the gas phase, an approximation of the CO_2 concentration in the liquid phase was calculated. The calculated CO_2 in the pore water at a given atmospheric CO_2 mixing ratio is shown in Figure 23 below. Based on the calculated CO_2 concentration under the maximal gas phase concentration (7600 ppm), the K_M value of CA would have to be below 0.3 mM to cause a competition for the active center of the enzyme. As long as the CO_2 concentration in the aqueous phase is not notably above the K_M value of CA, the rate of turnover will increase linearly with the increase of substrate concentration (in this case CO_2). This means that the enzyme is not saturated and some enzyme molecules still have free binding sites. All CO_2 concentrations in the aqueous phase were estimated to be well below the typical K_M value for CA and CO_2 and hardly any reported K_M value is below the highest CO_2 concentration that can be expected for the aqueous phase. Therefore, a competitive inhibition is regarded as highly unlikely.

The same selection criteria were applied for PEPCO and RubisCO, yielding medians of 2 mM (range of 0.8-3.7 mM) and 0.09 mM (range 0.08-1.4 mM), respectively. Badger and Bek (2008) report a similar value for RubisCO (0.1 mM). Thus, inhibition of PEPCO is unlikely as well, but RubisCO might be affected at higher CO_2 concentrations (see Figure 23). It has been shown that soils can contain significant amounts of RubisCO (Selesi et al., 2007; Videmšek et al., 2009; Salinero et al., 2009; KoilRaj et al., 2012; Nowak et al., 2015; Liu et al., 2016). If RubisCO is also contributing to the soil samples examined here, this inhibition would be part of the cause for the observed changes in OCS exchange at high CO_2 concentrations.

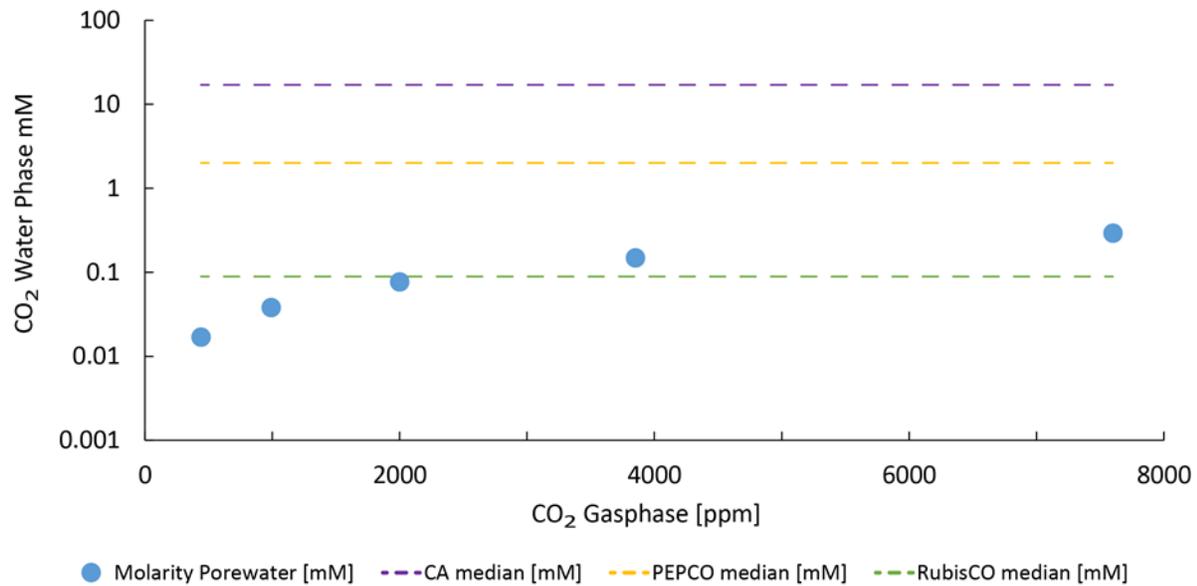


Figure 23 The K_M values of Carbonic Anhydrase (purple), PEPCO (yellow) and RubisCO (green) compared to the calculated CO_2 concentration in the water phase (blue dots). The expected water phase concentration was calculated from the known gas phase concentration following Henry's law. The K_M values are medians of data reported in the BRENDA database (Schomburg et al., 2002; www.brenda-enzymes.org)

4.3 Chloroform treatment demonstrates involvement of biotic processes

Fumigation by chloroform changed the exchange patterns and showed clear differences relative to untreated live soils. Despite the differences from soil to soil, some common trends were noticeable. Within the "optimal moisture range" all three soils that were examined under chloroform sterilization showed a shift towards OCS uptake with rising CO_2 mixing ratios. When compared with their live counter parts, OCS uptake at high CO_2 mixing ratios (2010/2000 to 3850/4030 ppm) was boosted for all three soils, in case of the "Mainz" soil under up to 7600/7520 ppm CO_2 . At lower mixing ratios (440/460 and 990/1010 ppm), uptake was reduced for "Mainz" and "Nördlingen sugar beet" and even switched to an emission (which declined with rising CO_2 mixing ratio) for "Nördlingen corn". At 7600/7520 ppm CO_2 uptake of "Nördlingen sugar beet" and the emission of "Nördlingen corn" was lower than that of their live counterparts.

Since treatment with chloroform vapor only kills a portion of the bacteria and fungi in soils (Ingham and Horton, 1987) and both production and consumption of OCS might be impacted, a quantification of the biotic share of OCS exchange with this method is not possible. However, as the physical properties of the soil remain the same, the strong change in exchange behavior demonstrates the involvement of biotic processes. It is also possible that killing the microorganisms in a soil does not stop all biotic processes: Maire et al. (2013) reported that respiratory enzymes can survive outside of living cells within soils, establishing an extracellular metabolism (EXOMET). This may also be possible for enzymes such as CA (uptake of OCS) or carbon disulfide hydrolase (production of OCS) stabilized on soil particles in a similar fashion. The fact that chloroform acts by lysis of cell membranes (Blankinship et al., 2014) increases the possibility of enzymes leaving the dead cells and establishing such an EXOMET. Also, despite the cells being dead, enzymes involved in the exchange of OCS might become more accessible, after the elimination of the diffusion resistance through the cell membrane. The duration of this effect would be dependent on the time required to degrade such enzymes, but Maire et al. (2013) have shown that some respiratory enzymes can be stabilized on soil particles for days.

Despite some inconsistencies, there seems to be a trend of increased uptake of OCS after chloroform vapor treatment. In conjunction with Ingham and Horton (1987) reporting chloroform vapor to be less effective against fungi than against other microorganisms (Table 6 in Ingham and Horton, 1987), this falls in line with other findings in this work which will be further discussed in Section 4.5.

4.4 Special exchange behavior of Nördlingen Sugar Beet and Suriname soil

(i) Suriname soil: The Suriname soil exhibited a more or less constant emission that decreased only at very low soil moisture. This behavior is not understood, but was observed already earlier (van Diest, 2007). This soil was found to be differed from all other soils in its organic content, carbon and nitrogen content, and pH (see Table 2). Interestingly, Whelan et al. (2016) also observed little variability in the OCS exchange of a rainforest soil when altering soil water content, while soils of other origins showed a strong reaction to changes in soil water content.

(ii) Nördlingen Sugar Beet soil: Compared to the other arable soils, the Sugar Beet soil showed an inverse reaction in OCS uptake to increasing CO₂ concentrations. Instead of a decrease of OCS uptake with increasing CO₂ concentration, an increase was observed for this soil. The abiotic properties of the Sugar Beet soil are rather similar to the other arable soils (see Table 2). The only prominent difference (especially to the other Nördlingen soil) is the fertilization practice. While the other three soils are conventionally managed, the Sugar Beet soil is organically fertilized. There is evidence that organically fertilized soil may contain more fungi than conventionally fertilized soil. Anastasi et al. (2005) found that both compost and vermicompost, typically applied for organic fertilization, contain very high amounts of fungi. This load of fungi was higher than or as high as the arable soils with the highest load reported by Luppi Mosca et al. (1976). Applying such compost as fertilizer to a soil will consequently also transport fungi into the soil. In contrast, several authors describe a negative impact of conventional (mineral) fertilizer on mycorrhizal fungi (Galvez et al., 2001; Oehl et al., 2004; Gryndler et al., 2006). Gryndler et al. (2006) also observed an increase of actinomycetes and arbuscular mycorrhizal fungi in organically fertilized soils, while the positive effect on saprotrophic fungi was not statistically significant. Griffiths et al. (1999) found that fungal biomass increased stronger than bacterial biomass when they loaded soil with organic carbon. Birkhofer et al. (2008) found that microbial biomass and activity of decomposer biota increased in comparison with soil that received only mineral fertilization, the effect being similar for bacterial and fungal biomass. Wallenstein et al. (2006) saw a decline of

the fungal to bacterial activity ratio in forest soils that received mineral fertilization and DeForest et al. (2004) saw a decrease of lignolytic enzyme activity in forest soil induced by mineral fertilization, indicating reduced activity by saprotrophic fungi. Esperschütz et al. (2007) found the highest fungal biomass in organically fertilized arable soils, when compared to conventionally or unfertilized arable soils. Bittman et al. (2005), however, observed a negative effect of organic fertilizing on hyphal length in grassland soils in comparison with unfertilized controls. This might be related to different land use type (grassland versus forest or arable soil). Thus, it is likely that the fungus to bacteria ratio within the “Nördlingen sugar beet” soil is higher than in the other arable soils, which were fertilized conventionally.

4.5 Fungi as dominant OCS consumers

Three trends in the presented measurements suggest that fungi are the dominant OCS consumers in the soils described in Section 2.2.1. (1) at optimal soil moisture, the one soil likely to have the highest fungi to bacteria ratio was the only one with a trend to stronger OCS uptake with increasing CO₂ (see Section 4.4 ii). (2) after chloroform vapor treatment at higher CO₂ concentrations there was stronger uptake or less emission of OCS in comparison to live (untreated) soils. Following Ingham and Horton (1987), fungi populations will be less reduced than bacteria populations. There are reports of bacteria producing OCS (Katayama et al., 1992; Smith and Kelly 1988; Jordan et al., 1995; Jordan et al., 1997; Smeulders et al., 2011), but no reports of fungi producing OCS. If the producing populations (bacteria) are suppressed more intensely by chloroform than the main consumers (fungi), a shift towards net consumption is expected. This is observed mainly at high CO₂ and supports the idea that OCS production by bacteria is boosted by higher CO₂ concentrations (see Section 4.2). The shift in the net exchange will be stronger at higher CO₂ concentrations because more OCS production than at low CO₂ concentrations will be taken away. (3) after antifungal treatment with nystatin, OCS emission was

stronger than from the same soils after antibacterial or no treatment (see Figure 10). This may be regarded to result from an inhibition of fungi, resulting in a decrease of uptake. This view is supported by the absence of reports about fungi producing OCS.

4.6 OCS exchange for organic layers and litter layer from needle forests

OCS exchange from the laboratory measurements of the Finland litter layer soil presented here match field OCS exchange measurements performed by Sun et al. (2017) at the site the samples were taken (SMEAR II site, Hyytiälä) surprisingly well, despite different measurement methods, experimental conditions and the fact that samples for this laboratory study were collected earlier. Figure 24 shows a comparison of OCS deposition velocity (uptake rate normalized by ambient OCS mixing ratio, see section 2.6). This good agreement suggests that,

(1) Laboratory measurements with soil chambers as performed in this study can adequately simulate processes at field sites

(2) The OCS exchange at the SMEAR II site is dominated by processes in the litter layer

All three organic soil samples from forests were almost exclusively OCS sinks, with U_{OCS} being much higher than P_{OCS} , especially at moderate soil moisture, a behavior which may change under elevated CO_2 concentration, however (see Sections 3.1 and 4.2). U_{OCS} and net uptake were a lot higher than for the agricultural mineral soil examined in this study. The litter layer sample from the Finland site (SMEAR II Station, Hyytiälä) had both significantly higher P_{OCS} and U_{OCS} than the two Waldstein samples from the organic layer (roughly 4-fold on average each). Like the good agreement of the presented laboratory exchange measurements with field data from Sun et al (2017) (see above) this suggests that the litter layer might be the most important layer for soil-atmosphere OCS exchange. Further, this is in good agreement with the experiment utilizing the

selective inhibitor Nystatin (see Sections 3.3 and 4.5), where a dominant role in OCS uptake by fungi was observed. There are important differences in the vertical distribution of a soils' fungal community. Lindahl et al. (2007) describe a vertical distribution of fungi in needle forest soil with saprotrophic fungi preferring the upper litter layer and mycorrhiza fungi the deeper litter layer and organic horizon. Dickie et al. (2002) report spatial variation in the abundance and distribution of mycorrhiza fungi with different groups of fungi preferring different depths in the litter layer and soil. Regarding the difference in U_{OCS} between the Finland litter layer sample and the Waldstein samples from the organic layer this suggests that saprotrophic fungi might take up more OCS than mycorrhiza fungi. The 20% difference in sink strength between the Waldstein soils with young spruce or blueberry understory might be due to one or both of the following two reasons:

(1) While both conifers and Ericaceae form mycorrhiza, conifers (like spruce) typically form ectomycorrhiza while Ericaceae (like blueberry) form a special type of mycorrhiza known as ericoid mycorrhiza (Cairney and Meharg, 2003, Czesnik and Eynard 1990). So the difference might be due to different groups of mycorrhiza fungi being present.

(2) The difference might also be due to the ratio of mycorrhiza to saprotrophic fungi. The most prominent difference between the Waldstein soil with blueberry understory and young spruce understory is their ammonium content. Mycorrhiza fungi are known as effective ammonium importers (Willmann et al. 2007). Consequently, the sample with the higher mycorrhiza content is to be expected to have the lower ammonium content because the ammonia pool is getting depleted by the mycorrhiza fungi. The Waldstein soil with spruce understory has much higher ammonium content, indicating less mycorrhiza to deplete its ammonium pool, yet its carbon content is higher than in the Waldstein Spruce sample. Likely, saprotrophic fungi and other non-mycorrhiza soil organisms make up the difference in carbon content. As the Waldstein Spruce sample has the higher U_{OCS} , this would be consistent with the hypothesis above (which is based

on the comparison of Finland litter layer and Waldstein organic layer uptake) that saprotrophic fungi contribute stronger to soil OCS uptake than mycorrhiza fungi.

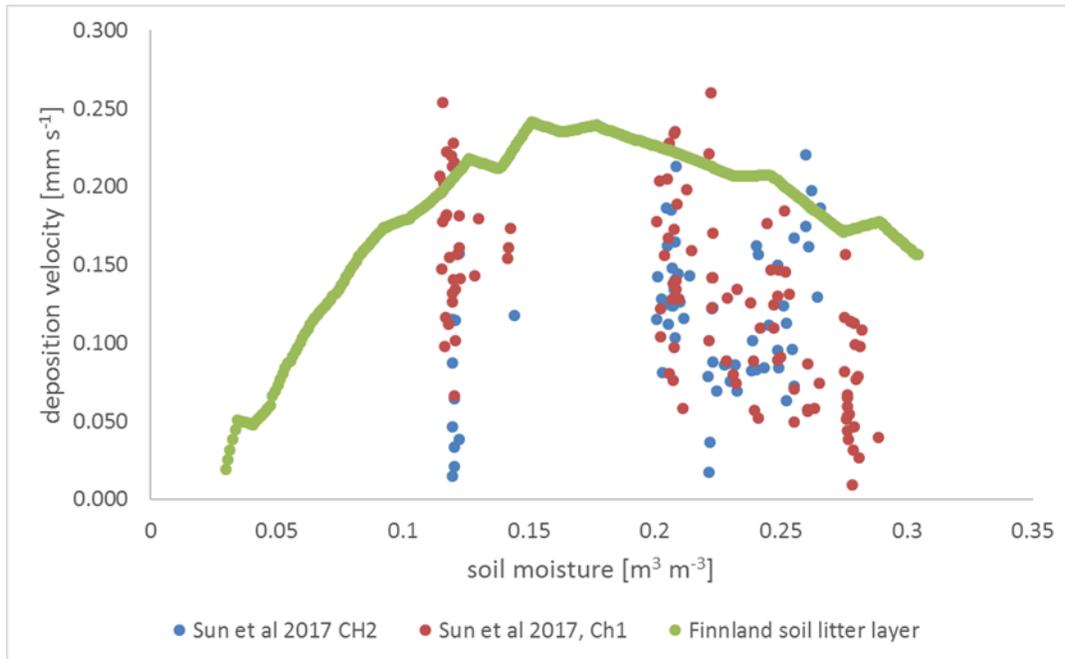


Figure 24 Field data from Sun et al. (2017) (blue and green dots) compared to lab OCS exchange measurements presented here. To compensate for varying OCS mixing ratios during the field measurements, data from the presented lab measurement and the data from Sun et al. (2017) were recalculated into deposition velocities.

4.7 The relationship of P_{OCS} and U_{OCS} to soil moisture

As P_{OCS} does not change significantly with soil moisture (see section 3.6), the changes in soil-atmosphere net OCS exchange must be driven by U_{OCS} . This suggests that the organisms responsible for OCS uptake are mainly active at medium soil moisture or that uptake processes are otherwise not possible at high soil moisture, i.e. due to limited diffusivity at high soil moisture as suggested by Van Diest and Kesselmeier (2008) or unavailability of oxygen. The organisms related to the production of OCS on the other hand must be active at nearly the full range of

tested soil moisture (except very low soil moisture). This implies that the influence of soil moisture discussed in Section 4.1 mainly applied to the uptake of OCS and less to the production.

4.8 Compensation points

All soils in this study showed a clear correlation between OCS exchange and ambient OCS mixing ratio. This is in good agreement with Kesselmeier et al. (1999) who have shown the uptake of OCS by soils to be linearly dependent on ambient OCS mixing ratio. In contrast to OCS consumption, which is proportional to the atmospheric mixing ratio (Kesselmeier et al., 1999) OCS production should be independent from atmospheric OCS mixing ratio. For the production processes from thiocyanate (Katayama et al., 1992, Katayama et al., 1998) and carbon disulfide (Smith and Kelly, 1988; Smeulders et al., 2011) no negative or positive feedback processes by OCS are known. At high moisture, where uptake rates are expected to be lowest (see below), exchange rates measured at high (1000 ppt) and low (50 ppt) ambient OCS mixing ratio are the most similar. The Mainz soil had especially close exchange rates at high soil moisture at all three measured OCS mixing ratios. The range of soil moisture at which exchange rates are similar is broader (70-100% MWHC) for the Mainz and Finland soils, while that range is rather narrow for the Waldstein soils (90-100% MWHC). Remaining differences between exchange rates at high and low ambient OCS mixing ratio at high soil moisture are probably due to not fully suppressed uptake at high soil moisture.

According to calculations the Waldstein soils and the Finland needle leaf soil are expected to act as sinks. With a CP between 460 to 510 ppt at about 20-30% MWHC, which is near the typical atmospheric OCS mixing ratio, the arable Mainz soil is expected to be a weak sink when compared to the forest soils. At moistures above and below that moisture range Mainz soil is expected to be a source as its CP is higher than the typical atmospheric OCS mixing ratio in these moisture

ranges. Seasonal fluctuations as well as nearby strong sinks or sources can modify local OCS mixing ratios, shifting a soil's expected OCS exchange behavior accordingly.

Some compensation points have been calculated in previous works (Lehmann and Conrad 1996; Kesselmeier et al., 1999) that compared to the compensation points calculated here (Figure 13). Lehmann and Conrad (1996) found very high compensation points when including all their measurements which implied that those soils would act almost exclusively as sinks at the soil moistures they measured OCS exchange for. When they compared measured OCS exchange rates for OCS free air to OCS exchange rates they predicted based on those compensation points the measured OCS production was only 2-8% of the predicted production. They proposed two mechanisms that could explain the discrepancy between their measured and projected values. The first was the existence of a threshold mixing ratio for OCS uptake, the second the existence of two different consumption processes that would be active at high or low ambient OCS mixing ratios and have different uptake rate constants. For this second mechanism Lehmann and Conrad 1996 recalculated compensation points using only exchange rates from experiments with the lowest ambient OCS mixing ratios they had performed. This resulted in considerably lower compensation points. Three of these compensation points (for the BW, BL and PBE soil in Lehmann and Conrad 1996) fall within the range of compensation points calculated for the soil measurements presented here and are shown in Figure 13. Only the compensation point for the RA soil was far outside the range of compensation points that were observed for soils in this study (approx. 11500 ppt at 18% MWHC, which is roughly tenfold the highest values calculated for soils in this study at the corresponding soil moisture) and is not shown in Figure 13.

Kesselmeier et al. (1999) determined the compensation point for one soil at one soil moisture for several temperatures. That soil is from the same sample site as the Mainz soil in this work. They found a temperature dependent range of compensation points (57 to 300 ppt atmospheric mixing ratio) that is in the same magnitude as the compensation point found for this soil at the

same soil moisture here (see Section 3.5 and Figure 13). However, the compensation points in the upper range in Kesselmeier et al. (1999) are about 100 ppt lower than the ones observed in Section 3.5. The lowest CP Kesselmeier et al. (1999) observed are about 300 ppt lower than the ones found in Section 3.5 for the same soil. That range is shown in figure 3 as a black bar. This difference might be due to changes in the microbial community that occurred during the 15 years that passed between the two samplings of the site, or due to differences in experimental method and instrumentation.

Compensation points were calculated based on flux data measured at mixing ratios that are low when compared to the measurements of Lehmann and Conrad (1996). The compensation points Lehmann and Conrad (1996) recalculated under the assumption that there are two different uptake processes at low and high atmospheric OCS mixing ratios, group reasonably well with the compensation points calculated from the OCS exchange data presented here. Though limited, this agreement of compensation points from Lehmann and Conrad (1996) with the compensation points from this work would support the second mechanism they suggested, as the atmospheric OCS mixing ratios at which the presented measurements were performed fall within the range of Lehmann and Conrad's (1996) low OCS mixing ratio measurements. The first mechanism they proposed is also supported by the data presented here, as only emission of OCS occurs at very low atmospheric mixing ratios (50 ppt) and the typical decrease in emission (often strong enough to switch to uptake) at medium soil moistures that always can be observed at higher OCS mixing ratios did not occur. Consequently, it seems likely that both mechanisms suggested by Lehmann and Conrad (1996) exist and should be considered in future soil-atmosphere OCS exchange observations. The presented data suggests that the threshold mixing ratio proposed by Lehmann and Conrad (1996) has to be higher than 50 ppt.

4.9 Comparison of dry and field fresh stored Mainz soil and to historic data

The OCS exchange behavior of Mainz soil followed a very similar pattern after dry storage and storage at moisture as found while sampling (“fresh”). For both, there was uptake of OCS at about 12% gravimetric soil water content which was reduced when the soil contained a higher or lower amount of water, gradually switching to emission of OCS at wet and very dry states as demonstrated in Figure 25. Exchange measurements made 6 years before by van Diest and Kesselmeier (2008) showed a very similar curve, but here uptake is stronger and is only reduced in the wet and very dry soil moisture range without switching to emission. This suggests two things: (1) Even when resampling after 6 years and after two different types of sample processing, the same mechanisms and drivers control the OCS exchange of the Mainz soil, producing very similar trends and patterns (2) Some changes occur both over time and induced by two different ways of sample treatment (storing “fresh” or air drying the sample before storage), illustrating the importance of consistent treatment and storage of samples that are meant to be compared to each other.

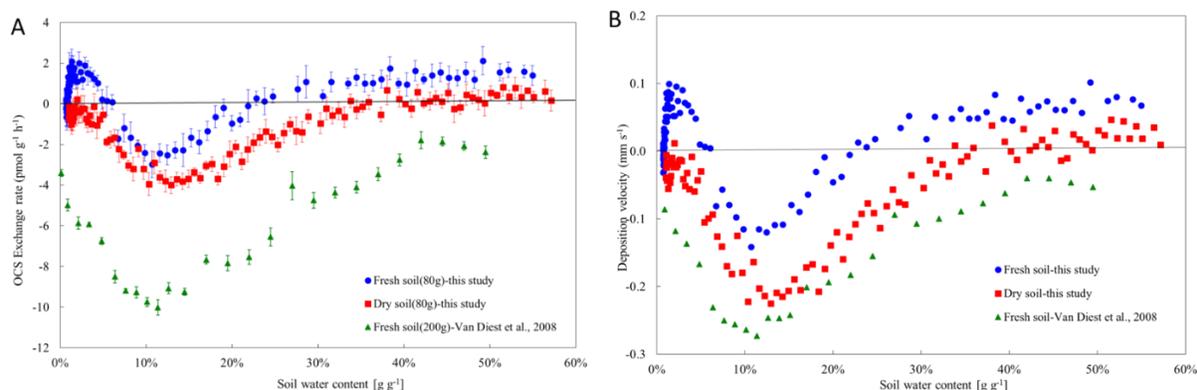


Figure 25 German arable soil from Mainz wheat ecosystem: A: COS uptake rates ($\text{pmol g}^{-1} \text{h}^{-1}$) in relation to the soil water content (gravimetric %) at 20°C for 80 g of soil per cuvette with the ambient COS mixing ratio of about 1000 ppt as inlet flushing air, compared with the results from Van Diest and Kesselmeier (2008) for 200 g of soil. B: Deposition velocities (V_d ; mms^{-1} ; normalized uptake rates) in relation to the soil water content (gravimetric %) at 20°C for 80 g of soil per cuvette. The recalculated data at 20°C for 80 g of soil from Van Diest and Kesselmeier (2008) was added in order to provide a comparison of the magnitude of the V_d .

4.10 Plant OCS exchange in comparison with soil exchange for spruce forests

Our OCS exchange rates for spruce forest soil as well as exchange rates for another spruce forest soil reported by Steinbacher et al. (2004) are low (about 1-5%) compared to the average fluxes over a spruce forest reported by Xu et al. (2002). Sandoval Soto et al. (2005) observed lower uptake by spruce trees in laboratory measurements. Here the soil uptake from this work amounts to about 26% of the plant uptake. Uptake rates of spruce forest soils and spruce are summarized in Table 7.

Table 7 OCS uptake by spruce forest soil and spruce trees in the presented measurements and reported in literature

Spruce forest soil uptake			
source	uptake [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	method	comment
This work	2.98	Chamber measurement, lab	Blueberry understory, max. uptake at optimal soil moisture
This work	3.42	Chamber measurement, lab	Young spruce understory, max. uptake at optimal soil moisture
Steinbacher et al., 2004	0.81	Chamber measurement, field	Average uptake, daytime
Spruce plant uptake			
Xu et al., 2002	93	Relaxed eddy accumulation, field	average
Sandoval Soto et al., 2005	12.6	Chamber measurements, lab	$600 \mu\text{mol m}^{-2} \text{s}^{-1}$ light

Considering field data, soil OCS fluxes for spruce forest soils appear to be small enough to be negligible when conditions for OCS uptake by plants are favorable. However, forest OCS uptake can be highly variable (Xu et al., 2002), because plant OCS uptake is controlled by stomatal conductance (Sandoval Soto et al., 2005). Stomatal aperture in turn is influenced by factors as temperature, light and water availability. Consequently, for spruce forests, when stomatal conductance is low due to lack of light, draught, or other unfavorable circumstances, soil OCS exchange might cover a significant portion of ecosystem OCS exchange due to plant OCS

exchange being reduced (see Kuhn et al., 1999). The contribution of soil exchange might be considered negligible for Spruce forest ecosystems when conditions for plant OCS uptake are favorable, considering (i) the ratio of spruce forest OCS exchange to Spruce forest soil OCS exchange, that (ii) there are no known OCS production pathways for plants and (iii) OCS production by plants has only been observed for plants infected with fungi (Bloem et al., 2012). However, when conditions are not favorable for OCS uptake, soil exchange can introduce a substantial uncertainty if not considered.

4.11 Outlook: Changes of plant OCS uptake at different CO₂ mixing ratios and their correlation to stomatal conductance

The decrease of stomatal conductance with increasing CO₂ mixing ratio observed in the presented experiments was expected. Stomata responding to ambient CO₂ mixing ratios and reducing aperture have been well recognized in literature (Morison 1987). Stomatal conductance has been shown to be one of the main controlling factors for OCS uptake by plants (Sandoval-Soto et al., 2005). This is further supported by the results of the Fusicoccin experiment presented in this work (see Section 3.8 and 4.12). Therefore, the close correlation of OCS uptake and stomatal conductance is in good agreement with the results of Sandoval-Soto et al. (2005). Further, it implies the changes in OCS uptake with increasing CO₂ mixing ratio are dominated by the changes in stomatal conductance. There is no indication of an uptake reduction beyond reduction by lower stomatal conductance. This is in agreement the report of Stimler et al. (2010) who found no cross inhibition between OCS and CO₂ in plant uptake. It also agrees with the comparison of K_M values of the key enzymes to the amount of CO₂ expected to be solved in water at the chosen ambient mixing ratios presented in Section 4.2. Sandoval-Soto et al. (2012) attributed the decrease of OCS deposition velocity under elevated CO₂ to competitive inhibition of OCS consuming enzymes by CO₂. This is in conflict with the current results. However, the trees

in Sandoval-Soto et al. (2012) were grown long term under elevated CO₂, which can lead to a reduction in RubisCO activity (Yelle et al., 1989, Vu et al., 1987).

The increase of OCS uptake at very high CO₂ mixing ratio by *Quercus robur*, *Sorbus aria* and *Fagus sylvatica*, even though stomatal conductance is still low, is curious. In comparison, the OCS uptake of *Zea mays* remains closely correlated to the stomatal conductance, both declining with increasing CO₂ mixing ratio. The three trees are C3 plants and *Zea mays* is a C4 plant. and it has been shown that C4 plants, especially Corn, can have less RubisCO than C3 plants (Ku et al., 1979). Yokoto and Canvin found varying RubisCO concentration in C4 species but found less RubisCO in Corn than in the C3 plants they examined. Therefore, it can be suspected that the difference in OCS uptake behavior between the three deciduous trees and Corn is connected to RubisCO. Assimilation of CO₂ or OCS by RubisCO comprises of two distinct steps. First, RubisCO must be carbamylated with one molecule of CO₂ (Lorimer and Pierce 1989). This step is distinct and the binding site different from the carboxylation/thiocarboxylation reaction that assimilates the CO₂ or OCS molecule. While OCS can be an alternative substrate for RubisCO, it is no substitute for this activating carbamylation step (Lorimer and Pierce 1989). Therefore, RubisCO has to be activated by CO₂ before it can assimilate OCS by thiocarboxylation. Therefore, more RubisCO activated by carbamylation might be available at high CO₂ mixing ratios effectively improving OCS uptake despite potential competition of OCS and CO₂ for the active center of the enzyme. The C4 plant corn, containing less RubisCO and relying more on other enzymes (CA and PEPCO) for OCS uptake, would profit less from that increased RubisCO activation. The meaning of OCS exchange behavior of *Quercus ilex* in this context is difficult to judge because data at the highest CO₂ mixing ratios where the increase was observed for other trees is not available. Mixing ratios of >4000 ppm CO₂ are so much higher than atmospheric values that it is unlikely this effect ever occurs under natural conditions.

4.12 Outlook: Fusicoccin treatment demonstrating stomatal conductance as a driver for OCS uptake and light independence of OCS uptake

Fusicoccin induces stomatal opening even under circumstances when stomata would normally be closed (Clauson-Kaas et al., 1944; Turner and Gratini 1969; Squire and Mansfield, 1972). As can be seen from the strong reduction of water vapor release, stomata of the *Quercus robur* branch closed in the dark before Fusicoccin application. After Fusicoccin application, the stomata did not close in the dark any more, as can be seen by the continued water vapor release. The remaining slight reduction in water vapor release was most likely induced by the temperature drop of about 3° C when the lights of the climate chamber were turned off. Prior to the Fusicoccin application, OCS uptake was effectively stopped by stomatal closure in the dark (see Figure 22). After Fusicoccin application, Stomata did no longer close in the dark. OCS uptake continued without any noticeable reduction in the dark. This demonstrates that OCS uptake by plants is controlled by stomatal conductance. This is in good agreement with Sandoval-Soto et al. (2005) who showed stomatal closure forced by abscisic acid effectively stopped leaf OCS uptake in the light.

Further, light independence has been shown by this Fusicoccin experiment, as the *Quercus robus* branch keeps taking up OCS in the dark. This light independence of plant OCS uptake has always been inferred because the enzymatic reactions of RubisCO, CA and PEPCO are independent of light, but to it seems to be the first time it has explicitly been shown experimentally. Furthermore, the use of Fusicoccin may open a new experimental procedure to test the effect of higher CO₂ concentration on trace gas exchange with plants limiting stomatal closure effects.

5. Conclusions and outlook

For the majority of soils examined (4 out of 5), a correlation between OCS exchange and CO₂ concentration as well as between soil moisture and OCS exchange has been found. All four arable soils showed a pattern going from OCS emission to uptake and finally to emission again when soils went from wet to dry state. The one soil (“Suriname”) with no clear relation between OCS exchange, soil moisture, and CO₂ concentration was very different in pH, organic carbon content, soil structure, and sample age. This behavior may be typical for such kinds of soil.

Results suggest that the net OCS exchange comprises biotic and abiotic processes. Fumigation with chloroform indicated that for the biotic component, OCS production exceeds consumption, especially at high soil moisture. The separation of the soil-atmosphere net exchange rates into production P_{OCS} and consumption U_{OCS} suggests that the correlation between soil moisture and OCS net exchange is mainly driven by U_{OCS} .

A comparison of estimated CO₂ concentrations in soil water with the K_M values of the enzymes involved in CO₂ and OCS uptake suggests that there is no competitive inhibition of PEPCO and CA at the CO₂ concentrations applied whereas inhibition of RubisCO cannot be excluded. Complementary plant OCS exchange measurements support the finding that competitive inhibition of the 3 key enzymes for OCS uptake is most likely not occurring.

The behavior of the organically fertilized “Nördlingen sugar beet” soil and preliminary experiments with selective sterilization agents suggested that fungi play a dominant role in biotic OCS uptake, as demonstrated for two completely different soil types, the arable “Mainz” and the tropical “Suriname” soils. Also, the composition of the fungal community in forest soils appears to play a major role in soil OCS uptake. Based on the ratio of the Finland soil uptake versus the Waldstein soil uptake, it can be concluded that saprotrophic fungi might take up more OCS than mycorrhiza fungi.

The presented data demonstrates that arable soils may switch between acting as sinks or sources, which puts in question the use of the overall net exchange signal over an ecosystem as a GPP proxy. Needle forest soils appear to act mainly as sinks, as suggested by the presented compensation point calculations. Accompanying soil exchange measurements must be included to check the significance of the soil exchange fluxes under the relevant field campaign conditions.

The observed switch of the arable Mainz soil from OCS uptake at medium soil moisture to emission at high and low soil moisture appears to be typical for arable soil and is in good agreement with prior publications.

High P_{OCS} and U_{OCS} from the litter layer Finland soil in comparison to P_{OCS} and U_{OCS} from organic layer Waldstein soil samples and the good agreement of the OCS net exchange rate measured for litter layer Finland soil with field data from Sun et al. (2017) show that soil-atmosphere OCS exchange is mainly driven by the litter layer at the Hyytiälä site.

The good agreement of the presented laboratory data for OCS exchange with field data from Sun et al. (2017) further confirms that results from laboratory chamber measurements as performed in this study can adequately simulate field conditions and results can be transferred to the originating site.

Comparison of exchange rates for spruce forests and spruce soils suggests that soil-atmosphere OCS exchange might be negligible in spruce forests when conditions for plant OCS uptake are favorable but might constitute a significant fraction of ecosystem OCS exchange when conditions are not favorable for plant OCS uptake, for example at night.

The hypothesis of Lehmann and Conrad 1996 that a second uptake mechanism at OCS higher and lower OCS mixing ratios and the existence of a threshold mixing ratio below which no uptake of OCS by soil occurs is supported by the data presented here. There is good agreement of the CP presented here with their CP recalculated based on this hypothesis. The threshold mixing ratio appears to be higher than 50 ppt.

Overall it has been shown that elevated CO₂ can have a considerable effect on OCS exchange with soils, typically increasing OCS emission or reducing OCS uptake, even though competitive inhibition of the OCS consuming enzymes now appears highly unlikely. The CO₂ concentrations regarded to be effective are within the range of typical soil pore concentrations.

Plant data could only be partially processed and evaluated to this point. Stomatal behavior seems to be the dominant control for OCS exchange. Further analysis and exploiting the full data set is likely to provide additional insights. Especially the use of the wilting toxin Fusicoccin may open further experimental approaches.

6. Literature

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Appendix A: Abbreviations

CA	Carbonic Anhydrase
MFC	Mass Flow Controller
OCS	Carbonyl sulfide
PEPCO	Phosphoenolpyruvate Carboxylase
RubisCO	Ribulose 1,5-Bisphosphate Carboxylase
RPV	Ring Poppet Valve (Swagelog) A spring based valve opening when a certain pressure (5 PSI in this case) is exceeded and closes again if the pressure falls below
GH ₂ O	stomatal conductance (for water vapor)
F _{cos}	plant OCS uptake
J	net OCS exchange (soil production –soil uptake)
U _{ocs}	soil OCS uptake
P _{ocs}	soil OCS production
CP	compensation point, the OCS mixing ratio at which OCS production and uptake by a soil are equal and the net exchange becomes 0
CO ₂	carbon dioxide
K _M	Michalis-Menten Constant, the substrate concentration at which 50% of an enzymes maximum substrate conversion speed is reached
ppm	parts per million
ppt	parts per trillion

