Microbial community responses determine how soil-atmosphere exchange of carbonyl sulfide, carbon monoxide and nitric oxide respond to soil moisture

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Research Article

Abstract

Carbonyl sulfide (OCS) plays an important role in the global sulfur cycle and is relevant for climate change due to its role as a greenhouse gas, in aerosol formation and atmospheric chemistry. The similarities of the carbon dioxide (CO_2) and OCS molecules within chemical and plant metabolic pathways have led to the use of OCS as a proxy for global gross CO_2 fixation by plants (gross primary production, GPP). However, unknowns such as the OCS exchange from soils, where simultaneous OCS production (P_{OCS}) and consumption (U_{OCS}) occur, currently limits the use of OCS as a GPP proxy. We estimated P_{OCS} and U_{OCS} by measuring net fluxes of OCS, carbon monoxide (CO) and nitric oxide (NO) in a dynamic chamber system fumigated with air containing different OCS mixing ratios [OCS]. Nine~~Several different~~ soils with different land use were rewetted and soil-air exchange was monitored as soils dried out to assess~~investigate~~ responses to changing moisture~~ levels~~. A major control of OCS exchange wa~~i~~s the total amount of available sulfur~~S~~ in the soil. P_{OCS} production rates were highest for soils at WFPS > 60~~-~~% and rates were negatively related to thiosulfate concentrations. These moist soils switched~~flipped~~ from ~~being~~ a net source~~s~~ to a net sink~~s~~ activity ~~of OCS~~ at moderate moisture levels (WFPS 15 to 37~~-~~%). For three soils we measured ~~By measuring CO and~~ NO and CO mixing ratios~~while fumigating soils~~ at different mixing ratios~~levels~~ of OCS~~,~~ and revealed that NO and potentially CO exchange rates ~~we could show that CO consumption and NO exchange~~ are linked to U_{OCS} at~~under~~ moderate soil moisture. High nitrate concentrations correlated~~Based on the~~ with maximum OCS release rates at high soil moisture.~~:CO flux ratio two different U_{OCS} processes could be separated.~~ For one of the investigated soils, moisture and OCS mixing ratio was correlated with different ~~we demonstrated changes in~~ microbial activity (bacterial 16S rRNA, fungal ITS RNA relative abundance) and gene transcripts of ~~and~~ red-like *cbbL* and *amoA* ~~genes that suggested shifts in the U_{OCS} processes with moisture and OCS concentration. This supports the view that~~

~~Ribulose-1,5-Bisphosphate-Carboxylase (RubisCO) plays an important role for U_{OCS} and demonstrates a link to the nitrogen cycle.~~

1 Introduction

Carbonyl sulfide (OCS) is the most abundant sulfur containing trace gas in the troposphere with a life time ion the order of years. OCS contributes to warming of the troposphere and cooling ofin the stratosphere, and both processes are considered balanced (Brühl et al., 2012). Plants simultaneously take up carbon dioxide (CO_2) and OCS by the enzymes contribution of the enzymes rRibulose-1,5-bBisphosphate-cCarboxylase (RubisCO) and pPhosphoenolpyruvate-cCarboxylase (PEPCO)., enhanced by Carbonic anhydrase (CA) enhances this uptake process, since it accumulates CO_2 intracellularly, (Protoschill-Krebs and Kesselmeier, 1992; Protoschill-Krebs et al. 1996). Thus, fluxes of OCS are closely related to gross Ppphotosynthesis, and represents the largest global OCS, i.e., sink with 0.73 to 1.5 Tg S a^{-1} (Sandoval-Soto et al., 2005). Thus, fluxes of OCS are closely related to gross CO_2 uptake during photosynthesis. Soils can act as both sources and sinks of OCS. While anoxic soils and wetlands are considered a global source for OCS of about 0.05 Tg a^{-1} (Watts, 2000), oxicupland soils are accounted as a sink for OCS of about 0.3655 Tg a^{-1} (Berry et al., 2013). OCS uptake in soils hais been consideredthought to be predominantly performeddominated by CA (Wingate et al., 2009). However, but there is some evidence that RubisCO of soil microorganisms might also play a role (Whelan et al., 2017; Kesselmeier et al., 1999, Meredith et al, 2018[b]). The microbial mechanisms underlying OCS production (P_{OCS}) and consumption (U_{OCS}) in soil, however, are not resolved and a topic to recent researchyet known (Ogée et al., 2016). In fact, current studies report that soils can switchflip between net OCS uptake and emission related either to soil moisture and/or soil temperature (Bunk et al., 2017; Whelan et al., 2016; Maseyk et al., 2014). Thus, anbetter understanding of environmental factors controlinginteracting with the soil microbial community is required for the predictiong of net soil OCS fluxes from the ecosystem to global scale.

~~The majority of~~ OCS can be~~is~~ produced~~released~~ by microbial decomposition of organic S compounds via thiosulfate (with minor amounts of CS_2; Smith and Kelly, 1988), and thiocyanate hydrolysis (Katayama et al., 1992). Nonetheless, alternative metabolic pathways for OCS production might occur in soil (Conrad, 1996). A recent study suggest thiocyanate as important precursor in microbial OCS production. However, there is no clear evidence if it is the only or main precursor in soil since it can also inhibit microbial OCS production (Katayama, et al., 1992).~~There is indication that also archaea are capable of OCS production via CS_2 hydrolase (Smeulders et al., 2011). OCS production from thiocyanate likely dominates in vegetated soils, due to thiocyanate which is released during decomposition of plant litter (Bunk et al., 2017; Kelly et al., 1993). Organisms that are known~~ S oxidizers bacteria that ~~to~~ utilize this pathway are *Thiobacillus thioparus*, *Thiohalophilus thiocyanatoxydans*, *Acinetobacter junii*, *Geodermatophilus obscurus*, *Amycolatopsis orientalis*, belonging to sulfur oxidizing bacteria (Katayama et al., 1992; Sorokin et al., 2006; Mason et al., 1994; Ogawa et al., 2016). Sulfate (Banwart and Bremner, 1976), S-containing amino acids (Banwart and Bremner, 1975), and other S compounds (Flöck et al., 1997; Lehmann and Conrad, 1996) can therefore act as precursors for microbial OCS formation. Additionally, an abiotic process, in which organic matter is degraded dependent on temperature and/or light might be of importance for P_{OCS} (Whelan et al., 2015).

Consumption of OCS can be linked to microbial pathways in soils that utilize ~~associated with utilization of~~ either CO_2 or bicarbonate (HCO_3^-) ~~substrates~~ by various microbial carboxylases~~.~~ ~~These enzymes can be differentiated and are similar to those found in plants~~ (Erb, 2011). CA~~arbonic anhydrase~~ reversibly catalyzes the hydration of gaseous CO_2, to bicarbonate (HCO_3^-) under neutral pH (Smith and Ferry, 2000). As an ubiquitous enzyme for exchanging and equilibrating CO_2, CA does~~it is~~ not only occur~~present~~ in ~~soils and~~ higher plants but also in algae and lichens, which may assimilate S~~the latter discussed to gain sulfur~~ from the atmosphere ~~this way~~ (Kuhn and Kesselmeier, 2000). ~~Within this context,~~ CA ~~has~~ also ~~been~~

~~shown to~~ irreversibly catalyze~~s~~ OCS to H_2S and CO_2 ~~in pure microbial cultures~~ (Ogawa et al., 2016; Protoschill-Krebs et al., 1995; Blezinger et al., 2000; Notni et al 2007). A recent study found a correlation of OCS exchange rates and $CO^{18}O$ with different forms of CA (Meredith et al., 2018[b]).

RubisCO occurs in plants and other photoautotrophs, ~~is present in all phototrophic tissues~~ and occurs in soil microbial chemolithoautotrophs~~cells and some autotrophic microorganisms in soils~~ (Badger and Bek, 2008). T~~, and t~~hus, RubisCO is also a candidate for OCS consumption. In plant leaves, stomatal control is the main regulator of OCS uptake~~, although elevated CO_2 may affect CA levels over the long term~~ (Sandoval-Soto et al. 2012). In soils, accumulating~~elevated~~ CO_2 mixing ratios may ~~levels~~ have ~~been discussed to have~~ the potential for ~~competitive~~ inhibition of RubisCO but not for the alternative enzymes by which soil organisms may uptake CO_2, such as CA or PEPCO (Bunk et al., 2017~~;). PEPCO similarly can fix HCO_3^- (~~Cousins et al., 2007) ~~and is present in both plants and soil microorganisms~~.

In addition to its co-metabolism due to its similarity with CO_2, OCS can be a direct source of sulfur and/or energy for some autotrophs and heterotrophs. Based on pure culture studies, *Thiobacillus thioparus* (Smith and Kelly, 1988; Kamezaki et al., 2016), fungal and bacterial strains belonging to *Trichoderma* (Masaki et al., 2016), and *Actinomycetales* (Ogawa et al., 2016)~~, respectively,~~ may~~could~~ degrade OCS. I~~nit~~~~ial~~ has been shown by Laing and Christeller (1980) that OCS acts as a competitive inhibitor for CO_2 uptake by RubisCO, where CO_2 and OCS compete for the active center of the enzyme as alternative substrates (Lorimer and Pierce, 1989)~~measurements of sulfur isotopic fractionation factors ($^{34}\varepsilon$) (Kamezaki et al., 2016) indicate the potential to estimate the OCS sink in soils using δ^{34}S measurements~~.

~~Additional clues to processes controlling uptake of~~ The OCS production process which has been found to correlate with the amount of nitrogen fertilizer (Kaisermann et al., 2018;

Melillo and Steudler, 1989) is~~n~~ still not understood and thus, it is still unknown if OCS consumption might be linked to the nitrogen cycle as well. In aerobic soils NO is predominantly produced by nitrifiers (e.g. Placella and Firestone, 2013). In addition, some methanotroph species fix carbon via RubisCO (Rasigraf et al., 2014, and references therein). Instead of RubisCO, ammonia oxidizing archaea utilize the hydroxypropionate/hydroxybutyrate cycle for aerobic CO_2 fixation (Könneke et al., 2014; Pratscher et al., 2011). Thus, there is evidence that microbial NO (and potentially CO) exchange might be linked to each other~~come from observing relationships with other gases consumed by soils, such as CO~~. Ammonia-oxidizing bacteria~~There is evidence that the sinks for CO and OCS are related to each other: (1) Nitrifiers~~ and methanotrophs may~~are capable of aerobic CO~~ co-oxidize~~ation~~ CO via ammonia monooxygenase (AMO) and methane monooxygenase (MMO) that likely is stoichiometrically correlated to ammonia oxidation (~~MMO, Bédard & Knowles, 1989; Jones & Morita, 1983;~~ Jones et al., 1984~~; Bender and Conrad, 1994~~)~~., whereas archaeal CO oxidizers are unknown (King and Weber, 2007).~~

There is some evidence that the CO and OCS consumption is coupled since various carboxydotrophic soil microorganisms exist. Soil ammonia oxidizers and methanotrophs are capable of CO co-oxidation via ammonia and methane monooxygenase (Bèdard & Knowles, 1989; Jones & Morita, 1983; Jones et al., 1984; Bender and Conrad, 1994). Aerobic carboxydotrophic bacteria and fungi can consume CO (King and Weber, 2007; Inman and Ingersoll, 1971). Inhibition experiments indicate that fungi might utilize CA for OCS consumption (Bunk et al., 2017). Archaeal carboxydotrophs are typically hyperthermophilic aerobes that are not common in temperate soils (King and Weber, 2007; Sokolovka et al., 2017). The energy conserved~~gained~~ from the oxidation of CO can be utilized for CO_2 fixation within the Calvin-Benson-Bassham (CBB) cycle via RubisCO (Ragsdale, 2004). ~~(2)~~ Anaerobes, such as acetogens, methanogens, and sulfate reducers that harbor ~~are able to~~

~~catalyze the oxidation of CO via carbon monoxide dehydrogenase (CODH) anaerobically within~~ the Wood-Ljungdahl pathway might also be capable to oxidize CO via carbon monoxide dehydrogenase, CODH~~of CO_2 fixation~~ (Davidova et al., 1993; Ragsdale 2004; Alber 2009)~~., that will also fix OCS. Also aerobic CO oxidizing bacteria are known which can consume CO (King and Weber, 2007). (3) Some fungi are able to consume CO (Inman and Ingersoll, 1971) and inhibition experiments indicate their role utilizing CA for OCS consumption (Bunk et al., 2017).~~ CO dehydrogenase can reduce OCS to CO and H_2S and the substrate affinity ~~for the substrate OCS, expressed as~~ K_M, is about 2.2 mM for OCS (Conrad, 1996).~~while for nitrogenase it is about 3.1 mM (Conrad, 1996).~~ While some enzymes consume only OCS (e.g. CA), others consume OCS and produce CO (e.g. CODH). Consistently, ~~Thus, it is assumed that the activity of different enzymes is expressed in the~~ OCS:CO ratios are correlated with CO:CO_2 ratios ~~. Sun and co-workers (2017) showed that OCS:CO_2 ratios are related to CO:CO_2 ratios~~ in a boreal forest (Sun et al., 2017). ~~Abiotic~~ CO production from abiotic~~, which is dependent on the temperature of~~ photodecomposition of organic matter (Conrad & Seiler, 1985), might be negligible~~occurs also~~ in soil~~s, but under dark incubation are expected to be small~~.

A key goal of our study was to explore whether simultaneous measurements of e.g. NO and CO and microbial activity by RNA-based approaches have the potential to indicate active metabolic pathways (e.g. CO_2 fixation via different enzymes). In turn, this information may provide insights into pathways of POCS and UOCS in a way that allows prediction of net OCS fluxes across a range of soils and moisture contents. Ultimately the ability to understand the role of soils in net ecosystem exchange of OCS is relevant to enable the estimation of canopy fluxes of OCS and their interpretation as a proxy for gross primary production, GPP (Campbell et al., 2017; Campbell et al., 2008; Blonquist et al., 2011; Berry et al., 2013).~~We expect that uptake of OCS via RubisCO will result in different OCS:CO fluxes compared to~~

~~the other enzymes discussed above. It has been shown by Laing and Christeller (1980) that OCS acts as a competitive inhibitor for CO_2 uptake by RubisCO, where CO_2 and OCS compete for the active center of the enzyme as alternative substrates (Lorimer and Pierce, 1989). A second process that can inhibit CO_2 uptake by RubisCO is described by Lorimer and Pierce (1989): if in the activation step RubisCO is thiocarbamylated by a molecule of OCS instead of being carbamylated by a molecule of CO_2 (which is distinct from the CO_2 molecule taken up in the carboxylation step, see Lorimer and Pierce 1989), the enzyme becomes catalytically incapable of taking up CO_2 or OCS in the carboxylation/thiocarboxylation step. According to differences in substrate affinity and reaction velocity a lower OCS than CO_2 concentration should be sufficient to result in competitive inhibition in CA reactions. For RubisCO the k_M ratio for OCS:CO_2 is only about 1 x 10^{-2} (Lorimer and Pierce, 1989) and therefore competitive inhibition at normal atmospheric levels for these gases seems unlikely. Thus, it is thought that the reversible process of thiocarbamylation can result in RubisCO remaining catalytically inactive for a certain time. By this mechanism elevated concentrations of OCS in soil pore space might be already sufficient to cause a perceivable inhibition of RubisCO. It can be hypothesized that the substrate affinity of RubisCO for CO_2 and OCS differs (see Lorimer and Pierce 1989).~~

Based on this approach, we investigated whether NO and CO exchange rates measured over a range of different moisture conditions and in different soils reveal the influence of soil moisture on the underlying microbial metabolisms of the net soil OCS exchange. For one of the investigated soils (an agricultural soil from Germany), gas exchange rates were linked to microbial activity of archaeal and bacterial ammonia oxidisers (AOA, AOB), and fungal activity based on RNA relative abundance of internal transcribed spacer (ITS).ITS RNA's half time is low since it is functionally not needed to the establishment of ribosomes, but can be considered as a general proxy for fungal protein biosynthesis (Žifčáková et al., 2016;

Baldrian et al., 2012). Additionally, quantitative real time polymerase chain reaction (qPCR) was applied for detection of the functional red-like *cbbL* gene encoding RubisCO (in nongreen algae and α and β-Proteobactereia, Selesi et al., 2005) and archaeal and bacterial amoA gene encoding ammonia monooxygenase. This study is based on the assumption that an increase in the numbers of rRNA and ITS RNA relative abundance reflects increased metabolic activity (Blazewicz et al., 2013; Rocca et al., 2015). Nonetheless, rRNA content is not always directly related to activity since it is relatively stable. ~~urther clues as to underlying processes can be gained through investigation of other gases. For example, there is evidence that the sinks for CO and the source for nitric oxide (NO) are related to each other: (1) ammonia oxidizing bacteria and methanotrophs can co-oxidize CO via AMO/MMO in soils that should stoichiometrically link CO consumption to ammonia oxidation (Jones et al., 1984). (2) In aerobic soils NO is predominantly produced by nitrifiers (e.g. Placella and Firestone, 2013). In addition, some proteobacterial methanotrophs are known to fix carbon via the CBB cycle (Rasigraf et al., 2014, and references therein). (3) Instead of RubisCO, ammonia oxidizing archaea utilize the hydroxypropionate/hydroxybutyrate cycle for aerobic CO_2 fixation (Könneke et al., 2014; Pratscher et al., 2011). Hence, OCS exchange rates should be linked to the CBB cycle of ammonia oxidizing bacteria (AOB) and methanotrophic bacteria (MTB). The simultaneous measurement of CO and NO exchange rates might therefore provide clues as to which microbial groups dominate the overall gaseous exchange in different soils.~~

~~A key goal of this work is to explore whether simultaneous measurements of e.g. CO and NO and microbial activity can indicate the operation of pathways (e.g. CO_2 fixation via different enzymes), that in turn can provide insight into pathways of P_{OCS} and U_{OCS} in a way that allows prediction of net OCS fluxes across a range of soils and moisture contents. Ultimately the ability to understand the role of soils in net ecosystem exchange of OCS is relevant to~~

~~enable the estimation of canopy fluxes of OCS and their interpretation as a proxy for gross primary production, GPP (Campbell et al., 2017; Campbell et al., 2008; Blonquist et al., 2011; Berry et al., 2013).~~

~~In this study, we investigated whether CO and NO exchange rates measured over a range of different moisture conditions and in different soils suggest how moisture influences underlying microbial metabolisms and the net soil OCS exchange. For one of the investigated soils (an agricultural soil from Germany), gas exchange rates were linked to microbial activity of archaeal and bacterial ammonia oxidizers (AOA, AOB), methanotrophic bacteria (MTB) and fungal activity based on relative abundance of internal transcribed spacer (ITS) sequences. Additionally, quantitative real time polymerase chain reaction (qPCR) was applied for detection of the functional red like *cbbL* gene encoding RubisCO and archaeal and bacterial *amoA* gene encoding ammonia monooxygenase. We present a conceptual understanding of OCS exchange from soil that links OCS production and OCS consumption processes to different CO_2 fixation pathways. Thus, our results are useful to predict under what conditions soil fluxes will be an important component of ecosystem OCS fluxes, which processes are predominant, and therefore impacting estimates of GPP based on net OCS flux.~~

2 Materials and Methods

2.1 Soil analysis

In total 11~~9~~ samples of topsoil (integrating a depth between 0-5 cm) were used, representing different soil types and land uses (see Table 1). To make a representative sample for each site, 9 individual subsamples were taken on a grid from within a 10 x 10 m area and homogenized. Samples were sieved to < 2 mm, hand-picked to remove roots, and stored at 4°C (for up to 6 months) prior to incubations. The field moist soil used for the incubations was analyzed for total sulfur (*S*) and thiocyanate (SCN^-) to link OCS production to substrate availability at the

start of the incubation experiments. Bulk soil sulfur content was determined on an elementar analyser (Vario EL, Elementar Analysesysteme GmbH, Germany). For thiocyanate measurement about 8 g of soil was extracted in 1 M sodium hydroxide (NaOH) solution, centrifuged and filtered to remove particulates. Thiocyanate concentrations (reported per gram dry soil) were determined colorimetrically using 50 mm cuvettes and adding chloramine-T-isonicotin acid as well as 1,3 dimethylbarbituric acid (Environment Agency, 2011). Absorption measurements were made at 600 nm using a photometer (DR3900, Hach Lange GmbH, Germany), calibrated based on a standard curve of diluted potassium thiocyanate from 1-5 mg L^{-1}. The blank for photometry analysis was subjected to the same color reactions as the samples using 1 M NaOH instead of sample extract. For ammonium (NH_4^+) and nitrate (NO_3^-) quantification 5 g soil have been extracted in 50 ml of 2 M KCl for 60 minutes and were filtered through a 604 ½ Whatman™ filter paper (GE Healthcare, Chicago, Illinois, USA). The filtered extracts were frozen at -20 °C until analysis with a flow injection analyzer (Quickchem QC85S5, Lachat Instruments, Hach Company, Loveland CO, USA).

2.2 Incubations

An automated dynamic chamber system was used to incubate soil at 25 °C in the dark (Behrendt et al., 2014). The system has 6 chambers, switching so that it is measuring one while flushing the other five. It also includes a soil-free reference chamber. Experiments of pseudo-replicates, which were representative for a 10 x 10 m area were run in series, with 3 technical replicates at any given time, for a given soil moisture. Each chamber was measured for 15 minutes and then flushed at a rate of 2.5 L min^{-1}.

At the start of each experiment/ ~~(~~run (for overview see Table 1), soil (~ 60 g) was moistened to saturation~~field capacity~~ (100 % water-filled pore space, WFPS) for most soils; 80 % WFPS for desert soils (D1 and D2 ~~samples~~) and placed into Plexiglas incubation chambers (inner

diameter 0.092 m, height 0.136 m). The composition of air entering the chambers (flow 500 mL min^{-1}) was adjusted by adding ~~ambient levels of~~ CO_2 (Westfalen, Germany~~~ 400 ppm~~) to a CO_2 free air stream using soda lime to reach ~ 400 ppm ± 8 ppm and a variable amount of OCS to "zero" air produced by a pure air generator (PAG 003, Eco Physics AG, Switzerland~~),~~ ~~CO_2 ambient~~. For practical reasons, different experiments were performed to test various controls on OCS fluxes. First, OCS fluxes were compared using soils from agricultural sites~~samples~~ – corn (A1 and A2), sugar beet (A3), and wheat (A4) as well as from a grassland site (A5), from sand deserts (D1 and D2), and from a natural and previously burned rainforest (F1 and F2) under ambient OCS mixing ratios (about 500 ppt). ~~A1 to A5, both 50 ppt and 1000 ppt of OCS were used. For samples D1, D2, F1, and F2 only one level of OCS (500 ppt) was used.~~ Second, for 3 soils NO and CO exchange rates were compared under 50 and 1000 ppt OCS fumigation using the fresh and 40°C dried mid-latitude cornfield soil (A1) Mainz, Germany and a soil sample originated from a spruce forest (F3) Sparneck, Germany. Data for OCS exchange for A1 are shown in the supplementary information. Third for only one site, a fresh mid-latitude cornfield soil (A1) also previously investigated for OCS exchange (Kesselmeier et al., 1999; Van Diest && Kesselmeier, 2008; Bunk et al., 2017) we stopped the incubation at selected moisture and subsampled for molecular analysis. During the incubations, sub-samples of this soil were taken at 4 different soil moistures flushed with OCS-free air (50 ppt). In addition, one sample at the moisture representing maximum OCS consumption under 1000 ppt OCS fumigation was taken for microbial analysis.~~The fluxes of gases (OCS, NO and CO) were calculated from the concentration difference between air exiting and entering the chamber and the mass flow rate. In all experiments reported here the inlet air contained no CO or NO, and the soil was treated with different OCS inlet mixing ratios of either 50, 500 or 1000 ppt, depending on the experiment (see Tab. 1).~~

For OCS, comparison of net fluxes measured using different levels of OCS in inlet air allows separate quantification of OCS production and consumption contributions to the net flux (KaisermannBehrendt et al., 20184). As the air entering the chamber is moisture-free, the soils dry out over time, allowing us to see how gas production and consumption changed with soil moisture. At the start and end of each experiment the gravimetric soil moisture (θ_g) was determined. Over the course of the experiment gravimetric soil moisture was determined by calculating the mass balance of evaporated water vapor (Behrendt et al., 2014).

For the comparison of results of soils that differ in texture, the gravimetric soil moisture was converted into percent of water filled pore space, $WFPS_{lab}$ as

$$WFPS_{lab}(t_i) = \frac{m_{soil}(t_i) - m_{soil}(t_s)}{m_{soil}(t_s)} \cdot \frac{100}{\theta_{sat}} \quad (1)$$

where θ_{sat} is the gravimetric soil moisture at saturationfield capacity, which was estimated by re-wetting the soil until the surface of particles were covered by a tiny film of water (see Bourtsoukidis et al., submitted). $M_{soil}(t_i)$ equals the dry mass of soil plus water at any given time point t_i and $m_{soil}(t_s)$ equals the dry mass of soil plus the residual mass of water at the end of the experiment.

As the inlet air always had ambient O_2 concentrations, the potential for anoxia in the wettest soils may have been reduced compared to what might be experienced in a field setting in soil. However, as the soils sat for a period before air flow was initiated, the first results may reflect anoxic conditions in the soils. Different experiments were performed to test various controls on OCS fluxes. First, OCS and CO fluxes were compared using soils from agricultural sites - corn (A1 and A2), sugar beet (A3), and wheat (A4), as well as from a grassland site (A5), from sand deserts (D1, D2), and from a natural and previously burned rainforest (F1, F2) under ambient OCS mixing ratios (about 500 ppt).

~~Second, soil CO and NO exchange rates were compared under 50 and 1000 ppt OCS fumigation using the 40 °C dried mid latitude cornfield soil (A1), Mainz, Germany. Data for OCS exchange for A1 are used from a separate study (Bunk et al., submitted). We additionally present here CO and NO exchange rates for these incubations of A1 soil and focus in their patterns in correlation to OCS exchange under 50 ppt and 1000 ppt OCS fumigation (for overview of experiments, see Tab. 1).~~

~~Third, for only one site (A1), a mid-latitude cornfield soil also previously investigated for OCS exchange (Kesselmeier et al., 1999; Van Diest & Kesselmeier 2008; Bunk et al., 2017; Bunk et al., submitted) we stopped the incubation at selected moisture contents and inlet OCS concentrations and performed intensive molecular analysis to see how the gas fluxes represented active microbial genes. During the incubations, sub-samples of this soil were taken at 4 different soil moistures fumigated with 50 ppt OCS. In addition, one sample at the moisture representing maximum OCS consumption under 1000 ppt OCS fumigation was taken for microbial analysis.~~

2.3 OCS, ~~CO,~~ NO and CO exchange rates

The selected outflow from the six soil chambers of the automated incubation system was connected to a commercial OCS/CO_2/CO/H_2O analyzer (907-0028, Los Gatos Research Inc., USA). Absorption peaks were detected at gas-specific spectral lines (OCS at 2050.40 cm^{-1} and CO at 2050.86 cm^{-1}). The instrument performs an off-axis integrated cavity output spectroscopy (OA-ICOS), a type of cavity enhanced absorption spectroscopy. In principle the absorption of a quantum cascade laser light by a trace gas~~es~~ is measured according the Bouguer Lambert Beer's law. For incubations of the agricultural soil (A1 fresh and 40 °C dried) and a soil sample from a spruce forest (F3)~~A4 soils~~, a NO_x analyzer was also connected to the collection line (42i-TL, Thermo Scientific, USA), and NO was detected via

chemiluminescence. NO standard gas (5 ppm, Air Liquide, Germany) was used for the calibration of the NO_x analyzer ~~and the accuracy and precision of the OCS analyzer was validated across another OCS instrument (Bunk et al., 2017)~~. The limit of detection was estimated based on the 3σ of the noise from the soil free chamber (LOD_{NO} = 0,15 ppb NO, LOD_{OCS} < 15 ppt and LOD_{CO} < 0.3 ppb). The precision and accuracy of laser spectrometers has been evaluated in detail elsewhere (Kooijmans et al., 2016).

In front of the inlet of both analyzers a nafion dryer (perma pure MD-110, Perma Pure LLC, USA) was installed. The exchange rate of each trace gas, J_{TG}, OCS, N~~C~~O, and C~~N~~O was calculated as

$$J_{TG}(c_{ref}, T_{const}, WFPS) = \frac{Q \cdot (c_{out} - c_{ref})}{M_{soil}} \quad (2)$$

where Q is the flow rate through the chamber (2.5 L min^{-1}), c_{out} and c_{ref} are the concentrations of each trace gas at the outlet of the soil chamber and soil free chamber (ng m^{-3}), respectively (Behrendt et al., 2014). M_{soil} equals the dry mass of soil after dried for 48h at 105 °C. The average and standard deviation of the fluxes were calculated for the last 5 points of each 15 minute interval the air exiting the soil was analyzed, over the entire time period during which the soil dried out. While the OCS mixing ratios measured were all above the limit of detection, the difference between OCS mixing ratio of incoming and outgoing air, especially under moderate to low soil moisture, was generally only a few parts per trillion. Therefore, it seems reasonable to set a threshold of detection (i.e. the minimum detectable rate of production or consumption based on the noise of the instrument). Similar to the definition of a limit of detection, we used 3 times the deviation of OCS mixing ratios measured from one soil chamber to define this threshold and converted it into a OCS exchange rate of about ± 1.09 pmol g^{-1} h^{-1}.

2.4 Extraction of RNA and amplicon sequencing

~~For more detailed process understanding, microbial measurements, NO, CO and OCS fluxes were measured under two levels of OCS in inlet air only for a single soil, the mid-latitude cornfield from Mainz, Germany (A1). Soils were sampled at 95 %, 33 %, 21 % and 7 % $WFPS_{lab}$ with 50 ppt of OCS (to minimize OCS consumption compared to OCS production) in inlet air for amplicon sequencing and qPCR analysis to analyze which microbial groups might be involved in the OCS production, P_{OCS}. Under 1000 ppt OCS only one sub-sample for sequencing at 21 % WFPS was taken, since it is quite well known that maximal OCS consumption in agricultural soils mostly occurs under moderate soil moisture conditions.~~ A commercial RNA extraction kit (RNA Power Soil, MOBIO, USA), involving bead beating at 6 m s^{-1} for 30 s for cell disruption (FastPrep, MOBIO, USA), was used for RNA extraction of about 1 g soil. RNA has been eluted in 100 µl nuclease-free water and further cleaned with a commercial kit for RNA (RNeasy Power Clean Pro Clean-Up Kit, MOBIO, USA). Quality and quantity of purified nucleic acids were analyzed by agarose gel electrophoresis (1 % w/v), nanodrop (ND-2000, Thermo Fisher Scientific, USA) and Qubit (Thermo Fisher Scientific, USA). RNA integrity and quantity were analyzed by agarose gel electrophoresis (0.5 % w/v) and Qubit analysis, after DNase treatment (DNase Max Kit, MOBIO, USA). Subsequently, cDNA was produced with random hexamer primers (Roche) and SuperScript III Reverse Transcriptase (Invitrogen, Karlsruhe, Germany). Ribosomal 16S rRNA and ITS genes were amplified for the V3-V4 region (Klindworth et al, 2013) and ITS3F-4R region (White et al., 1990), respectively, from cDNA. Amplification and sequencing library preparation were performed for MiSeq Illumina platform in Macrogen Inc. (Seoul, South Korea).

2.5 qPCR archaeal and bacterial *amoA* gene and for red-like *cbbL* gene

The abundance of archaeal and bacterial *amoA* functional marker gene encoding ammonia monooxygenase (AMO) was measured by real-time polymerase chain reaction (qPCR), with the crenamo23f/crenamo616r (Tourna et al., 2008) and amoA1F/amoA2R primers (Rotthauwe et al, 1997), respectively. The red-like *cbbL* functional marker gene encoding RubisCO large subunit type IA was quantified with cbbLR1F and cbbLR1intR primers (Selesi et al., 2007). The total reaction volume of 20 µl consisted of 2 µl DNA (1 ng μl^{-1}) or cDNA (diluted 1/50), 0.4 or 0.6 µM of primer (archaeal and bacterial *amoA*, respectively), 1 x Power SYBR Green PCR MasterMix (Invitrogen, Karlsruhe, Germany), performed in a qPCR cycler (StepOnePlus™, Applied Biosystems, USA). Reactions were performed in triplicate, and cycling parameters were set to 10 min at 95 °C for initialization, and 40 cycles of denaturation at 95 °C for 30 s, annealing for 30 s at 54 °C (archaeal *amoA*) or 60 s at 55 °C (bacterial *amoA*) or 30 s at 55 °C (*cbbL*), and 30 s at 72 °C for elongation, followed by fluorescence measurement. Melting curves were measured in the range of 60 to 95 °C in 0.3 °C increments. Standard curves were created from 10-fold dilutions of purified plasmids containing the respective gene of interest as described previously (Catão et al., 2016). Archaea and bacterial *amoA* standard curves had 87.5 % and 67.1 % efficiency~~, respectively~~ and 0.93 and 0.97 coefficient of determination (R^2), respectively. The abundance of red-like form of Rubisco was calculated from 10-fold dilutions standard curve produced from purified DNA of *Sinorhizobium meliloti* obtained from DSMZ (number 30135), with 84.8 % efficiency and 0.99 coefficient of determination (R^2).

2.6 Sequence analysis

The RNA relative abundance was used as proxy for microbial activity in this study. Before sequence analysis was performed with a standard QIIME pipeline, paired-end reads of 300 bp were merged with PEAR (Zhang et al, 2014), with maximum lengths of 500 or 550 bp for 16S

rRNA and ITS, respectively and cleaned with PrinSeq (Schmieder & Edwards, 2011). Specific criteria were used to proceed the analysis only with high-quality reads in terms of sequence confidence: mean phred over 25 (probability that the base assigned by the sequencer is at least 99%), trim quality window of 50 (space of nucleotides scanned for quality at each time); minimum length of 200 bp; removal of artificial duplicates obtained during sequencing and only 1% of bases, which were not recognized as ATGC, were allowed (Schmieder & Edwards, 2011). Pre-cleaned sequences were analyzed with QIIME Version 1.9.1 (Caporaso et al., 2010), following *usearch61* chimera (sequences that can be artificially created during amplification of DNA molecules for the sequencing) screening, and operational taxonomic units (OTUs) picking process was performed by the *uclust_ref* method. Chimera checking, OTU picking and OTUs taxonomy assignment of representative OTUs was based on Greengenes taxonomy database 13.8 version for 16S rRNA (McDonald et al, 2012) and ITS UNITE 12.11 version for ITS (Abarenkov et al. 2010). Biome table was exported to *.tsv* and used for calculations in R (version 3.3.1) or Igor Pro 7. For graphical representation, overall description of taxa is presented as the normalized relative abundance of the counts (from Qiime pipeline) of sequences assigned to that taxa divided by the total amount of sequences obtained after cleaning steps for each sample. Similarly, only the first hit of classification (from blast approach), with highest bit score and lowest e-value was considered. The count of reads classified per species above was normalized per the total of cleaned reads and expressed per million reads.

3 Results

3.1 OCS exchange for ~~various soils~~ rewetted and dried-out soils under ambient (500 ppt) OCS

After wetting stored soils to 80-100 % WFPS ~~(field capacity)~~, all agricultural soils (A1 to A5)~~except the sugar beet soil (A3)~~ produced OCS, with rates of production declining as the soil dried out. At ~37 % $WFPS_{lab}$, these soils switched~~flipped~~ to a state of net OCS consumption (Fig. 1a). Around 15 % $WFPS_{lab}$ OCS exchange rates increased again to a local maximum (in some cases again net producing OCS) at about 10 % $WFPS_{lab}$ before they finally declined to zero exchange under completely dry conditions. The cornfield soil~~s~~ (~~A1 and~~ A2)~~,~~ produced ~~the most OCS, up to 2 and~~ 13 pmol g^{-1} h^{-1}, followed by the 4.7 pmol g^{-1} h^{-1} from the grassland soil (A5) and 3.8 pmol g^{-1} h^{-1} OCS from the wheat field soil (A4), respectively. For the sugar beet soil (A3), OCS fluxes were < 1.09 pmol g^{-1} h^{-1} (undetectable) or negative (net OCS uptake) in the range from 65 % to 15 % $WFPS_{lab}$ but increased to a production of 1.5 pmol g^{-1} h^{-1} at about 10 % $WFPS_{lab}$. The ~~A4~~ soil from a wheat field had an almost identical OCS exchange profile to the cornfield soil (A1). The grassland soil (A5) produced up to 5 pmol g^{-1} h^{-1} OCS and was the only agricultural~~A~~ soil that emitted OCS > 1.09 pmol g^{-1} h^{-1} within the range of moderate soil moisture. Both~~,~~ rainforest soil samples (F1 and F2)~~ rainforest samples~~ exchanged OCS above detection levels only at very high and low soil moisture; both acted as small net sinks for ~~as~~ OCS in between (Fig. 1b). The two ~~sandy~~ desert soils~~,~~ (D1 and D2, sand content ≥ 90% determined according to ISO 11277)~~,~~ produced up to 3.3 to 9.56 pmol g^{-1} h^{-1} at high soil moisture, with fluxes declining as the soil dried out (Fig. 1c).

We measured thiocyanate in soil extracts at start of the dry-out experiments where high P_{OCS} was observed, because a pathway of thiocyanate hydrolase has been proposed for OCS production (P_{OCS}). Thiocyanate concentrations for the desert soils was very low, below detection for D1 (< 0.5 mg kg^{-1}, ~~Environment Agency, 2011;~~ grey point in Figure 2), and only 0.65 mg SCN^- kg^{-1}~~, but still detectable~~ for D2. For all other soils, thiocyanate concentrations ranged between 0.87 and 12.04 mg SCN^- kg^{-1}. For all soils except the agricultural soil (A2~~,3 (~~

not used in curve fitting), an increase in thiocyanate concentration coincided with a ~~logarithmic~~ decrease in the maximum observed OCS exchange rate at WFPS > 37 ~~–~~%, $OCS_{max,HM}$ (see Fig. 2). The maximum OCS exchange rate and thiocyanate concentration for the agricultural soil (A2, green circle) are considered as an outlier, possibly due to the release of thiocyanate from fine roots during the sieving procedure.

While the agricultural soils (A) and forest soils (F) ~~soils~~ showed similar patterns that included a second increase in OCS production at bellow~~about~~ 10 ~~-~~% $WFPS_{lab}$, desert soils (D) ~~soils~~ only produced OCS. The different behavior for OCS exchange from desert soils may be related to differences in soil properties: desert soils (D) ~~soils~~ are characterized by high pH (carbonate contents of 1.89 to 0.55 ~~-~~% for D1 and D2 soils respectively) and high amount of total sulfur (0.13 to 3.74 ~~–~~%). Highest NO_3^- concentrations from a desert soil (D2) and a cornfield soil (A2) correlated with largest net OCS exchange rates (see Table 1). The high NH_4^+ correlated with low maximum OCS exchange rate at start of the experiment, respectively. ~~owever, thiocyanate levels are non-detectable or very low. The availability of thiocyanate is negatively correlated to the overall magnitude of OCS fluxes (see Section 4.1), in particular the ability to net produce OCS at WFPS > 37 %.~~

3.2 ~~Bacteria and f~~Fungal~~i~~ activity correlated with~~involved in~~ P_{OCS} and U_{OCS} from a mid-latitude cornfield soil (A1) ~~soil over~~under the range of soil moistur~~different OCS fumigation (sequencing)~~

The highly conserved 16S rRNA gene reflects differences in bacterial and archaeal populations. Overall, the sequencing approach did not result in significant differences in 16S rRNA ~~transcript~~ relative abundance for bacterial classes for the cornfield soil (A1) ~~soil~~ fumigated at 50 versus 1000 ppt OCS at moderate soil moisture (Fig. 3~~a~~). In contrast, for ITS

RNA ~~transcripts the~~ relative abundance of Ascomycota (p-value = 0.006) indicated these were significantly more active under 1000 ppt OCS compared to 50 ppt OCS, which could suggest their importance for OCS exchange (Fig. 4). ~~and Basidiomycota (p-value = 0.034) indicated these were significantly more active under 1000 ppt OCS compared to 50 ppt OCS, indicating their importance for OCS exchange (Fig. 3b).~~ Within the phylum of Ascomycota the largest difference in RNA relative abundance from 50 ppt to 1000 ppt OCS resulted for the class Sordariomycetes (p-value = 0.029). Within the phylum Basidiomycota (p-value = 0.034) the largest difference in RNA relative abundance from 50 ppt to 1000 ppt OCS was observed for the class Cystobasidiomycetes (p-value = 0.009), also significantly more abundant in the OCS 1000 ppt samples. For the phylum Zygomycota the RNA relative abundance decreased from 50 ppt to 1000 ppt OCS (p-value = 0.035).

3.3 Effect of [OCS] on NO release rate~~fumigation on CO exchange~~

For the investigation of the microbial groups involved in OCS production and consumption, we studied simultaneous OCS, NO (as a proxy for nitrification) and CO exchange for a fresh and 40 °C dried cornfield soil (A1) at 50 ppt and 1000 ppt OCS (see supplementary information). The maximum NO release rate for the 40 °C dried cornfield sample (Fig. 5a) was 726.9 pmol g^{-1} h^{-1} at 50 ppt OCS and 1102.7 pmol g^{-1} h^{-1} at 1000 ppt OCS at 37% $WFPS_{lab}$, whereas for the fresh sample NO release rates were substantially lower (Fig. 6d). The soil sample from the spruce forest Sparneck, Germany (F3) released maximal NO of 5579.5 pmol g^{-1} h^{-1} at 50 ppt OCS and 7159.4 pmol g^{-1} h^{-1} at 1000 ppt OCS and 41% $WFPS_{lab}$ (Fig 5b), respectively. The observed increase of NO release rate at 1000 ppt OCS compared to 50 ppt OCS suggests that microbial groups involved in the nitrogen cycle (e.g. nitrifiers), which utilize CA and RubisCO, might had contributed to simultaneous exchange of NO and OCS under moderate soil moisture. Interestingly, at 1000 ppt OCS its OCS release rate was

lower (indicating OCS consumption increased) and coincided with low CO release compared to 50 ppt OCS under moderate soil moisture regime (see S. 2).~~mid-latitude cornfield soil (A1) at 50 ppt OCS fumigation OCS exchange rates reached up to 2 pmol g^{-1} h^{-1} at 50 % $WFPS_{lab}$ and at 5 % $WFPS_{lab}$ (green squares, Fig. 4a). Even if the soil was fumigated with 1000 ppt OCS, net OCS production at 7 and > 60 % WFPS was still observed. Under 1000 ppt OCS fumigation at 21% $WFPS_{lab}$ (red squares, Fig. 4a), net OCS uptake was observed, with exchange rates up to −2.4 pmol g^{-1} h^{-1}. Interestingly, lowest OCS release (indicating OCS consumption increased) and lowest CO release under 1000 ppt OCS fumigation occurred simultaneously under moderate soil moisture regime, indicating that CO consumption relative to production increased (see Fig. 4b). This indication of an increased CO uptake and OCS uptake under moderate soil moisture and 1000 ppt OCS fumigation guided us to the hypothesis that another enzyme than CA might contribute to simultaneous exchange of CO and OCS under moderate soil moisture.~~

3.4 Effect of OCS fumigation on the ~~RNA relative abundance of~~ archaeal and bacterial *amoA* ~~gene~~ and red-like *cbbL* gene transcripts (qPCR) from a mid-latitude cornfield soil (A1)~~and NO exchange~~

The change in 16S rRNA ~~transcript~~ relative abundance for bacteria (sequencing) could not resolve significant differences for a cornfield soil (A1)~~ soil~~ fumigated at 50 versus 1000 ppt OCS at moderate soil moisture (see Section 3.1). Hence, qPCR assays have been used for the specific quantification ~~of RNA relative abundance~~ of the AOB and AOA *amoA* and red-like *cbbL* gene transcripts. For the fresh ~~air dried A1~~ soil sample from a cornfield (A1) 34 AOB *amoA* transcripts per nanogram extracted DNA have been detected at 95% $WFPS_{lab}$ with a continuous increase up to 221 transcripts per nanogram extracted DNA at 7% $WFPS_{lab}$, all at 50 ppt OCS, respectively (see Fig. 6). 2,193 AOA *amoA* transcripts per nanogram extracted

DNA, have been detected at 33% $WFPS_{lab}$ with a continuous increase up to 39,494 transcripts at 7% $WFPS_{lab}$ at 50 ppt OCS (see Fig. 6). For red-like cbbL (RubisCO) 13,463 transcripts per nanogram extracted DNA have been detected at 95% WFPSlab with a continuous increase up to 45,033 transcripts per nanogram extracted DNA at 7% WFPSlab, all at 50 ppt OCS, respectively (see Fig 6).~~, AOB *amoA* RNA relative abundance is very low. AOB *amoA* decreased under 1000 ppt OCS (red point) compared to 50 ppt OCS (bright green point) at 21 % $WFPS_{lab}$ (see Fig. 5). For AOA *amoA* the RNA relative abundance increased under 1000 ppt OCS (purple point) compared to 50 ppt OCS (dark green point), but was not significant. Interestingly, the maximum AOB *amoA* RNA relative abundance under 50 ppt occurred at about 21 % $WFPS_{lab}$, whereas the maximum AOA *amoA* RNA relative abundance occurred at about 7 % $WFPS_{lab}$. At 21 % $WFPS_{lab}$, the red-like *cbbL* (encoding the CO_2 fixation enzyme RubisCO) RNA relative abundance , was lower (N=5, $p < 0.05$) under the 1000 ppt OCS fumigation treatment (red diamond) compared to the 50 ppt OCS treatment (green diamond) at 21 % $WFPS_{lab}$. For both OCS fumigations at 50 and 1000 ppt net release of NO, which can be used as proxy for nitrification, followed a similar pattern over the dry-out experiment than the AOB *amoA* RNA relative abundance. At 1000 ppt OCS fumigation the net release of NO was larger compared to 50 ppt OCS fumigation, and thus OCS fumigation seems to affect NO release rates and thereby nitrification.~~

4 Discussion

4.1 Interpretation of ~~Explaining~~ patterns of OCS exchange for ~~various soils~~ rewetted and dried-out soils under ambient (500 ppt) OCS

OCS is produced by the degradation of various S compounds. Thiocyanate has been considered as an important precursor for OCS (e.g. Conrad, 1996). Thus, it is likely that the

OCS production rate is correlated with the concentration of thiocyanate as a dominant intermediate of organic S compound degradation. Lehmann and Conrad (1996) added sodium thiocyanate to soil samples and found an increase in OCS production. Nonetheless, there is indication that also organic compounds might be precursors of OCS in soil (Smith and Kelly, 1988; Kelly et al., 1993). In our study, all vegetated soils (i.e. not D1 and D2 desert soils) contained significant amounts of thiocyanate that likely were produced during decomposition of plant material (Bunk et al., 2017; Kelly et al., 1993). In two tropical forest soils, thiocyanate and OCS fluxes were at or close to detection limits. Over a range of moisture conditions, these soils consume any OCS produced and provide a (barely detectable) sink for OCS from the atmosphere (Whealan et al., 2016; Sun et al., 2017).~~is reported in literature to be an important precursor for OCS (e.g. Conrad, 1996), thus, it can be expected that the OCS production rate should be related to the amount of thiocyanate as a dominant product of decomposition of organic sulfur compounds. Lehmann and Conrad (1996) added sodium thiocyanate to soil samples and found an increase in OCS production. In this study, all vegetated soils (i.e. not D1 and D2 desert soils) contained significant amounts of thiocyanate that likely was produced during decomposition of plant tissue (e.g. compiled by Bunk et al., 2017; Kelly et al., 1993). In the two tropical forest soils very low in overall S content, thiocyanate and OCS fluxes were at or close to detection limits. Over a range of moisture conditions, these soils consume any OCS produced and provide a (barely detectable) sink for OCS from the atmosphere (Whelan, et al., 2016; Sun et al., 2017; Bunk et al., submitted). The desert soils, although very low in thiocyanate, contained high bulk S, likely in the form of inorganic sulfur compounds, such as calcium sulfate or sodium sulfate. In deserts such enrichments of salts are the result of long term dry deposition (Michalski et a., 2004). These crusts promote the abundance of sulfur metabolizing microbes in a few mm thick crusts on the topsoil as reported from Wierzchos and co-workers (2011). These microbes might be able to produce OCS from sulfate (Banwart and Bremner, 1976) or other S-containing precursors~~

~~(Banwart and Bremner, 1975; Flöck et al., 1997, Lehmann and Conrad, 1996), and thus may have high rates of OCS production that do not depend on organic S availability. The absence of an OCS uptake mechanism in desert soils under moderate soil moisture might be explained by low concentrations or inhibition of RubisCO through high pH and the presence of carbonate (Lorimer and Pierce, 1989). Also very low amounts of organic matter might limit the abundance and activity of heterotrophs, such as fungi, which are also involved in OCS uptake (Ogawa et al., 2016).~~

The desert ~~In all~~ soils (D1 and D2), although exhibiting low thiocyanate concentrations, contained high bulk S, likely in the form of inorganic S compounds. In deserts such enrichments of inorganic salts are the result of long term dry deposition (Michalski et al., 2004). Microorganisms might be able to produce OCS from sulfate (Meredith et al., 2018[a]; Banwart and Bremner, 1976) or other S-containing precursors (Banwart and Bremner, 1975; Flöck et al.,1997; Lehmann and Conrad, 1996), and thus may have high rates of OCS production that do not depend on organic S availavility. The positive OCS net fluxes from desert soils (D1 and D2) at 500 ppt OCS suggest that OCS consumption in these soils is, if at all present, Low amounts of organic matter in these soils might limit the abundance and activity of heterotrophs, such as Actinobacteria (Ogawa et al., 2016). An alternative explanation is the inhibition of RubisCO through high pH and the presence of carbonate (Lorimer and Pierce, 1989). Both inorganic and organic S availability control OCS production rates in general (e.g. Meredith et al., 2018[a]; Banwart and Bremner, 1976; Banwart and Bremner, 1975; Flöck et al., 1997; Lehmann and Conrad, 1996), but rates of OCS consumption are controlled by different parameters (e.g. Kaisermann et al., 2018). And thus, net soil OCS exchange and its relation to moisture is not linear dependent on further controls.

~~OCS production is lower at higher soil moisture, even with increasing thiocyanate concentrations, indicating that maybe also other precursors, like organic carbon compounds, are needed for an efficient breakdown of sulfur compounds. There is indication from a purified enzyme study for thiocyanate hydrolysis that at > 40 mM thiocyanate an inhibition by the substrate was observed (Katayama et al., 1992). Both inorganic and organic S availability control OCS production rates in general, but rates of OCS consumption are controlled by different parameters. This may mean that net soil OCS exchange and its relation to moisture are not easily predicted.~~

There is already evidence that OCS exchange correlates with total nitrogen content (Kaisermann et al., 2018). In our study the highest nitrate concentrations correspond to maximum OCS net exchange under high soil moisture. This is in agreement with a nitrate fertilization study in which substantial increase of OCS net fluxes from forest soils was the consequence (Melillo and Steudler, 1989). The correlations of NO_3^- and NH_4^+ concentration with OCS net release rate at start of the experiment suggest that microbial nitrogen cycling is connected to OCS exchange.

4.2 Fungal activity correlated ~~The role of bacteria and fungi involved~~ with ~~in~~ P_{OCS} and U_{OCS} from a mid-latitude cornfield soil ~~the~~ (A1) ~~soil~~ over the ~~whole~~ range of soil moisture ~~under different OCS fumigation (sequencing)~~

Carbonic anhydrase is thought to be the most important enzyme involved in OCS uptake (Bunk et al. 2017). Masaki and co-workers (2016) concluded that fungal species may contribute differently to OCS exchange in soils, although some were net consumers of OCS, ~~since~~ pure cultures from strains of *Umbelopsis*/*Mortierella* sp. were net producers of OCS.

In our study, we found a significant difference in ITS RNA relative abundance for several fungi when OCS in ambient air was changed from 50 to 1000 ppt, indicating fungal sensitivity to OCS. Recent studies suggest that fungi containing CA might be responsible for OCS uptake (Ogawa et al., 2016; Bunk et al., 2017). In addition, enzymes involved in different CO_2 fixation pathways, including the CBB cycle, hydroproprionate/hydroxybutyrate cycle (HP/HB), anaplerotic reactions of heterotrophic microorganisms (PEPCO), or the Wood Ljungdahl pathway might play a role for OCS. For example, using a specific inhibitor for CA leads to changed OCS flux (Kesselmeier et al., 1999). A possible explanation for the large differences in OCS exchange among the various soils investigated here might be a niche separation (here soil moisture) of gene expression and activity maxima under different moisture conditions for different OCS-converting enzymes: At high soil moisture the OCS production by hydrolysis of organic S compounds might be the dominant process, while at moderate soil moisture consumption of OCS by CO_2 assimilation might be the predominant process. Under moderate soil moisture we found a lower activity of Zygomycota and Tremellomycetes at 1000 ppt compared to 50 ppt OCS, whereas both Sodariomycetes (Ascomycota showed highest RNA relative abundance of overall fungi in the mid-latitude cornfield soil, A1) and Cystobasidomycetes exhibited an increased metabolic activity (see Fig. 4).~~However, in addition to fungi the importance of phototrophs (algae) for $CO^{18}O$ and OCS exchange was demonstrated (Sauze et al., 2017).~~

~~In our study we found a significant difference in ITS transcripts relative abundance for several fungi when OCS in ambient air was changed from 50 to 1000 ppt, indicating fungal sensitivity to OCS. Recent studies suggest that fungi containing CA might be responsible for OCS uptake (Ogawa et al., 2016; Bunk et al., 2017). In addition, enzymes involved in different CO_2 fixation pathways, including the CBB cycle, hydroxypropionate/hydroxybutyrate cycle (HP/HB), anaplerotic reactions of heterotrophic~~

~~microbes (PEPCO), or the Wood Ljungdahl pathway might play a role for OCS exchange as already investigated by the use of 6-ethoxy-2-benzothiazole-2-sulfonamide (EZ) as a specific inhibitor for carbonic anhydrase (Kesselmeier et al., 1999). A possible explanation for the large differences in P_{OCS} and U_{OCS} among the various soils investigated here might be a niche separation (here: soil moisture) of different enzymes: At high soil moisture the hydrolysis of organic S compounds to produce OCS might be the dominant process, while at moderate soil moisture consumption of OCS with CO_2 fixation might be the predominant process. Under moderate soil moisture we found a lower activity of Zygomycota and Tremellomycetes under 1000 ppt compared to 50 ppt OCS, whereas Sordariomycetes (Ascomycota showed highest RNA relative abundance of overall fungi in A1 soil) and Cystobasidomycetes showed an increased activity, respectively (see Fig. 3). Our results are supported by a study which found that in agricultural soils, where the lignin content of organic matter is typically low, Ascomycota are the key decomposers (Ma et al., 2013). Under low soil moisture other enzyme processes, such as the CS_2 hydrolase pathway for OCS production from archaea, which might be more resistant to desiccation, could be responsible for net OCS production (Smeulders et al., 2011), while consumption rates decline at low soil moisture.~~

Carbonic anhydrase is not a single enzyme but rather a group of 5 different families (α, β, γ, δ and ζ). A recent study suggest that Actinobacteria contain a CA-like gene, to which also OCS hydrolase are similar (Ogawa et al., 2016). Thus, these bacteria may contain a hydrolase that might be specialized to uptake OCS. The importance of phototrophs (eukaryotic algae) for OCS exchange was already demonstrated (Sauze et al., 2017). There is evidence that different CAs and likely other enzymes are involved in the OCS exchange (Meredith et al., 2018[b]).~~There is indication that β-CA and α-CA differ in their OCS hydrolysis rates (Ogawa et al., 2013, Ogawa et al., 2016; Ogée et al., 2016). However, the different families of CA are not really clustering into metabolically and phylogenetically distinct groups but rather show a~~

~~complex distribution based on their evolution in fungi, bacteria and archaea (Smith et al., 1999). In CO_2 fixation~~ CA is well known to act as an ~~"~~"upstream amplificatory"~~"~~ for e.g. RubisCO and PEPCO~~epCO~~. Due to similarity of the OCS and CO_2 molecules, it seems reasonable that for OCS consumption ~~in chemical pathways of OCS consumption~~ the roles of RubisCO and PEPCO~~PepCO were~~ might have been underestimated. There might be not only a bulk k_{cat} and K_m (Ogèe et al., 2016), but rather multiple parameters for diverse types of CA (Meredith et al., 2018[b]) and maybe even for other enzymes such as RubisCO (this study) necessary to fully understand and model the microbial OCS production and consumption from soils.~~In theory, the ubiquitous CA should result a uniform response of soil moisture, with a single optimum function as modeled in Ogée et al., (2016). Hence, a more complicated pattern in OCS exchange as observed in this study is more likely the result of an ensemble of enzymes with maximum activities at distinct soil moisture ranges. Within such an ensemble we want to point out that CA irreversibly catalyzes OCS to H_2S and CO_2 (Ogawa et al., 2016; Protoschill-Krebs and Kesselmeier, 1992; Protoschill-Krebs et al., 1995, 1996; Blezinger et al., 2000; Notni et al., 2007). Hence, the pattern in activity of different fungal genera under moderate soil moisture might be caused by differences in tolerance/inhibition or even utilization of H_2S.~~

4.3 Effect of [OCS] ~~fumigation~~ on NCO release rate~~exchange – similarity to N_2O:NO ratio?~~

While in other studies the OCS production and consumption are disentangled by utilizing different inlet mixing ratios (Kaisermann et al., 2018), we introduce a new concept of measuring different gases, such as NO release rate (as a proxy for nitrification), simultaneous to OCS exchange rates to better understand which microbial groups are involved in OCS production and consumption. Interestingly under moderate soil moisture conditions, where

lowest OCS net release at 1000 ppt OCS occurred (see S. 1), maximum NO release rates were detected. Under moderate to low soil moisture NO net production is predominantly accepted to originate from nitrification (e.g. Oswald et al., 2013). NO release rates increased under elevated OCS fumigation, which agrees with our results. Based on the correlations with NH_4^+ and NO_3^- concentrations (section 4.1), we hypothesize that microbial groups involved in the nitrogen cycle (e.g. nitrifiers and potentially denitrifiers) are involved in the OCS exchange. Interestingly, at 1000 ppt OCS its release was lower (indicating OCS consumption increased) and coincided with low CO release compared to 50 ppt OCS under moderate soil moisture (see S. 2). It is worth to note the correlation of OCS and CO exchange rates (see supplementary information S. 2), but given the lack of CO ambient mixing ratios at the inlet and the lack of CO dehydrogenase activity measurement, we cannot fully explain that result. ~~OCS fluxes from litter samples incubated in the laboratory have been measured (Bunk et al., submitted) and are in good agreement with a field study at Hytiälä, Finland (Sun et al., 2017). Since in our incubations CO was scrubbed, we decided to reanalyze the field dataset from Sun and co-workers (2017, doi:10.5281/zenodo.322936) in a similar way to express averaged OCS:CO ratio over $WFPS_{field}$ moisture classes. The OCS:CO ratio shows a clear optimum function under moderate and high soil moisture (grey optimum function, Fig. 6). For the A1 agricultural soil we found a maximum activity of Sordariomycetes (Ascomycota) and Cystobasidomycetes under moderate soil moisture during fumigation with 1000 ppt OCS (where maximum OCS consumption was detected). Since we found a decrease in RNA relative abundance for Tremellomycetes (Basidomycota) and Basidomycota are known to play the key degraders in forest soils for lignin-rich litter (Blackwood et al., 2007), we hypothesise that they contribute to OCS and CO exchange at elevated soil moisture. At such elevated soil moisture from 40 – 60 % WFPS OCS consumption was detected (Bunk et al., submitted), even confirmed to be correlated to abundance of fungi (Sauze et al., 2017) and is corresponding to our second maximum in OCS:CO ratio (see Fig. 6).~~

~~To the best of our knowledge we could not find any process involving only CA that would result in this distinct pattern by simultaneously affecting the uptake of OCS and CO. However, for alternative enzymes, e.g. RubisCO and PepCO, that have been shown to be at least partly involved in OCS exchange (Kesselmeier et al., 1999; Lorimer and Pierce, 1989), a simultaneous consumption of CO and OCS seems possible. Our results (fumigation performed at 1000 ppt OCS) also point out that the correlations of microbial activity to OCS consumption are difficult to interpret, since both a microbial production of OCS as well as a utilization of OCS as sulfur and/or energy source can affect the microbial activity and overall differences are small. The 2 distinct optima in OCS:CO ratio might be related to different kinetics of CO and OCS consumption for distinct microbial groups at about 46 % and 21 % WFPS (see Fig. 6), respectively. This differentiation of 2 OCS consumption processes based on CO and OCS metabolism is supported by different patterns of OCS consumption rate coefficients k_{OCS} reported from Bunk et al., (submitted) and by a simultaneous increase in OCS uptake rates and bacterial and fungal abundance in alkaline soils (Sauze et al., 2017).~~

~~All of our incubations were performed aerobically and CH_4 was scrubbed from the inlet air. Therefore, our analysis focused only on a selected subset of microbial groups that might be involved in the OCS exchange. We excluded methanogenic archaea and acetogens (where only a low number of sequences was obtained). Under anaerobic conditions in the field, they can use the Wood Ljungdahl pathway for CO_2 fixation and CODH and thus might also be involved in OCS uptake at high soil moisture. There is evidence that ammonia oxidizing bacteria and methanotrophs can co-oxidize CO aerobically (Jones et al., 1983; Jones et al., 1984), and *Methylococcus capsylatus* and *Methylocaldum szegediense O-12* utilize the CBB cycle for carbon fixation (Rasigraf et al., 2014). Since in our study the inlet air was free of methane, we could not observe the activity of methanotrophs. However, under elevated CH_4 conditions in the field methanotrophs should consume CO and might also be involved in OCS~~

~~exchange via RubisCO under moderate soil moisture range. Hence, in our laboratory incubations ammonia oxidizing bacteria (also utilizing RubisCO and consuming OCS) might be the dominant CO consumers and NO net producers. NO net production, commonly accepted to originate from nitrification (e.g. Oswald et al., 2013) under low to moderate soil moisture, increased under elevated OCS fumigation, which is in agreement with our results. Thus, we suggest the use of OCS:CO ratio to separate the activity of different microbial groups (AOB, methanotrophs, Sordariomycetes and Cystobasidomycetes versus Zygomycota and Tremellomycetes) in a similar way than the N_2O:NO ratio is used to separate the activity of nitrifiers and denitrifiers (Davidson et al., 2000).~~

4.4 Effect of OCS fumigation on the 16S rRNA relative abundance of archaeal and bacterial *amoA* ~~gene~~ and red-like *cbbL* gene transcripts (qPCR) ~~and NO exchange~~

Despite the evidence for nitrogen-dependent OCS exchange, the mechanosms are not understood (Kaisermann et al., 2018; Melillo and Steudler, 1989). Fungi are considered as relevant OCS consumers utilizing CA over the whole range of soil moisture (Bunk et al., 2017). However, there is increasing evidence that OCS consumption is not performed by a single metabolic process (Sauze et al., 2017; Meredith et al., 2018[b]; our study). Our data suggest that indeed CA plays an important role for OCS exchange, but also for further enzymes (e.g. RubisCO) being involved in CO_2 assimilation. At high soil moisture, anaerobes such as, acetogens, methanogens, and sulfate reducers, might had been active and might had been capable of catalyzing the oxidation of CO via CODH via the Wood-Ljungdahl pathway to fix CO_2 (Davidova et al., 1993; Ragsdale, 2004). Since the incubations were performed under oxic conditions and CO production was observed from the soil (inlet air was free of CO), the contribution of CO consumption via the Wood Ljungdahl pathwy from anaerobic pockets at elevated soil moisture range might had been underestimated. Under moderate soil

moisture, reduced CO production may be predominantly attributed to the activity of aerobic CO_2 assimilating microorganisms (Bèdard & Knowles, 1989; Jones & Morita, 1983; Jones et al., 1984; Bender and Conrad, 1994) with minor importance of the aerobic CODH pathway (Conrad et al, 1981). Our study suggests that under moderate soil moisture prokaryotic autotrophs, Sordariomycetes (Ascomycota) and Cystobasidomycetes were dominant OCS consumers in the mid-latitude agricultural soil (A1). Our study highlights how gene expression information on enzymes involved in CO_2 fixation combined with the simultaneous assessment of NO and CO as well as OCS exchange are useful for understanding the complex microbial controls on net OCS exchange from soils.

We restricted the discussion of the microbial groups involved in OCS consumption to fungi since the involvement of bacterial groups would have required a more specific approach such as stable isotope probing to prove their involvement. The strength of our study is the proven correlations of OCS net exchange to NH_4^+, NO_3^- (at start of the incubations), NO exchange and functional genes (AOB and AOA amoA and red-like cbbL RubisCO over drying out at 50 ppt OCS).~~For the experiments with the A1 soil, the only difference was the level of OCS fumigation, which was either 50 ppt or 1000 ppt. While there is evidence that theoretically for a 10^6 higher level of CO_2, RubisCO can be saturated (Bunk et al., 2017), the level of OCS fumigation applied in this study should not lead to saturation of either CA or RubisCO. Reported K_M values of CA for OCS are 0.039 mM (extracted from pea leaves, Protoschill-Krebs et al., 1996) and 1.86 mM (from *Bos Taurus*, Haritos and Dojchinov, 2005). The only reported K_M value of RubisCO for OCS reported in literature we know of is 1.8 mM (extracted from spinach, Lorimer and Pierce, 1989). To competitively inhibit an enzyme, the concentration in the soil water would have to at least reach the enzyme's K_M value for that substrate. However, following Henry's Law and the according constants as published in Sander (2015) the soil water concentration will only be 2.57 x 10^{-8} mM. Therefore,~~

competitive inhibition of either enzyme must be considered highly unlikely (see Fig. 7). It also has been shown that the thiocarbamylation by a molecule of OCS can inhibit CO_2 fixation via RubisCO and the enzyme is incapable for both, CO_2 and OCS uptake (Lorimer and Pierce, 1989). The simultaneous decrease of AOB *amoA* gene and *cbbL* gene at 21 % WFPS for A1 soil under 1000 ppt OCS fumigation seems likely to be caused by thiocarbamylation. Under a continuous OCS fumigation the thiocarbamylation step of RubisCO inhibits the carboxylation/thiocarboxylation step (Lorimer and Pierce, 1989) and thereby the main carbon assimilation of AOB and methanotrophs. This might result a reduced activity of AOB and methanotrophs utilizing RubisCO which was detected as decrease of AOB *amoA* under 1000 ppt OCS fumigation. This reduced activity might explain the decrease in RubisCO which was observed in this study under 1000 ppt OCS fumigation.

Although the increase of AOA *amoA* RNA relative abundance at 1000 ppt OCS compared to 50 ppt OCS under 21 % $WFPS_{lab}$ was not significant, it indicates that AOA might outcompete AOB and produce more NO without consuming CO (King and Weber, 2007) under 21 to 7 % $WFPS_{lab}$. This is consistent with a recent study reported the higher transcriptional activity for AOA *amoA* under such low soil moisture from a dryland soil, suggesting that available moisture might act as niche separation for AOA and AOB (Behrendt et al., 2017). A similar interaction of the sulfur and nitrogen cycle was discovered already in a study which reported OCS exchange from soils under fertilization with ammonium nitrate (Sauze et al., submitted). Nitrifying and methanotrophic organisms are also capable of metabolising other compounds such as CO (Bender and Conrad 1994).

5 Conclusions

Fungi are considered ~~accepted~~ as dominant microbial OCS consumers in literature, which may utilize ~~utilizing~~ CA over the whole range of soil moisture (Bunk, et al., 2017). However, there is increasing evidence that OCS consumption is not performed by a single metabolic process (Kaisermann et al., 2018; ~~Bunk et al., submitted;~~ Sauze et al., 2017; Meredith et al., 2018[b], this study). Our data suggest that indeed CA plays an important role for OCS exchange, but the role of other enzymes involved in CO_2 fixation might have been underestimated. At high soil moisture creating anoxia, acetogens, methanogens and sulfate reducers are capable of catalizing the oxidation of CO (Davidova et al., 1993; Ragsdale, 2004). Our study suggests that under moderate soil moisture autotrophs (e.g. AOB), Sordariomycetes (Ascomycota) and Cystobasidomycetes are likely the dominant OCS consumers in the mid-latitude agricultural soil (A1). Our study highlights that simultaneous assessment of enzymes involved in CO_2 assimilation and simultaneous assessment of NO and potentially CO as well as OCS exchange is useful for disentangling the complex microbial controls of net OCS exchange from soils. Our study is the first assessment of the environmental significance of different microbial groups producing and consuming OCS by various enzymes other than CA. A combination of stable isotope probing with ^{32}S-labelled OCS plus metagenomics is required to prove our conclusions that further enzymes beyond CA are involved in OCS conversion. Our study is a first important step towards the understanding of the mechanism of microbial OCS consumption and production in soils. ~~Distinct maxima in the OCS:CO ratio support the molecular data and all together point towards the importance of RubisCO from AOB and methanotrophs for OCS consumption under moderate soil moisture regimes.~~

~~It is known that at high soil moisture acetogens, methanogens, and sulfate reducers are capable of catalyzing the oxidation of CO via CODH anaerobically via the Wood-Ljungdahl pathway to fix CO_2 (Davidova et al., 1993; Ragsdale, 2004). Since the incubations were~~

~~performed under aerobic conditions and CO production was observed from the soil (inlet air was free of CO), the contribution of CO consumption via the Wood Ljungdahl pathway from anaerobic pockets at elevated soil moisture range might be underestimated. Under moderate soil moisture, reduced CO production is mainly attributed to activity of AOB and methanotrophs (Bédard & Knowles, 1989; Jones & Morita, 1983; Jones et al., 1984; Bender and Conrad, 1994) with minor importance of the aerobic CODH pathway (Conrad et al., 1981). Our study suggests that under moderate soil moisture autotrophs (AOB and methanotrophs), Sordariomycetes (Ascomycota) and Cystobasidomycetes are dominant OCS consumers in the A1 mid latitude agricultural soil. We discuss the role of Zygomycota and Tremellomycetes (Basidomycetes) as additional important OCS consumers under elevated soil moisture in lignin rich organic horizons in forest soils. This study highlights how metabolic information related to enzymes involved in CO_2 fixation, inferred because we were able to simultaneously assess CO and NO as well as OCS exchange, are useful for disentangling the complex microbial controls on net OCS exchange from soils. Our study is the first assessment of the environmental significance of different microbial groups producing and consuming OCS by various enzymes other than CA.~~

Data availability. Raw sequencing data were deposited in the NCBI SRA accession number SRP121207, BioProjectID PRJNA415548. Data for trace gas release are stored in a database (http://bexis2.uni-jena.de/) and are available on request.

Competing interests. The authors declare that they have no conflict of interest.

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Tab. 1 Soil properties and experimental conditions~~ummary of soil samples and experimental conditions~~ used for analysis. Note that NO~~CS~~ and CO exchange rates were measured only for ~~fluxes for F3, F4, F5 and~~ A1, A1dry and F3 soils at 50 ppt and 1000 ppt OCS, respectively. ~~are presented in a separate study including the compensation points (Bunk et al., submitted)~~. Temperature for all experiments was 25°C.

~~Soil ID~~	~~Location~~	~~Coordinates~~	~~Vegetation cover~~	~~OCS [pmol g h^{-1}]~~	~~CO [pmol g h^{-1}]~~	~~NO [pmol g h^{-1}]~~	~~pH [1]~~	~~S [%]~~
~~*50 ppt OCS ‚zero air' || 400 ppm CO_2 ‚ambient'*~~								
~~A1~~	~~Mainz, GER~~	~~(49.951°N/ 08.250°E)~~	~~Corn~~	~~+~~	~~+~~	~~+~~	~~7.6*~~	~~0.03*~~
~~*500 ppt OCS 'ambient' || 400 ppm CO_2 ‚ambient'*~~								
~~D1~~	~~Bahariyya, EGP~~	~~(28.362°N/ 28.860°E)~~	~~-~~	~~+~~	~~-~~	~~-~~	~~8.3~~	~~0.13~~
~~D2~~	~~Waxxari, CHI~~	~~(38.705°N/ 87.414°E)~~	~~-~~	~~+~~	~~-~~	~~-~~	~~8.3~~	~~3.74~~
~~F1~~	~~Canarana, BRA~~	~~(13.077°S/ 52.377°W)~~	~~rainforest natural~~	~~+~~	~~-~~	~~-~~	~~4.6~~	~~0.02~~
~~F2~~	~~Canarana, BRA~~	~~(13.079°S/ 52.386°W)~~	~~rainforest burned~~	~~+~~	~~-~~	~~-~~	~~4.5~~	~~n. d.~~
~~A1~~				~~+~~	~~+~~	~~+~~		
~~A2~~	~~Baldingen, GER~~	~~(48.865°N/ 10.462°E)~~	~~corn~~	~~+~~	~~-~~	~~-~~	~~7.1*~~	~~0.03*~~
~~A3~~	~~Baldingen, GER~~	~~(48.866°N/ 10.866°E)~~	~~sugarbeet~~	~~+~~	~~-~~	~~-~~	~~7.2*~~	~~0.04*~~
~~A4~~	~~Baldingen, GER~~	~~(48.867°N/ 10.467°E)~~	~~wheat~~	~~+~~	~~-~~	~~-~~	~~7.7~~	~~0.03~~
~~A5~~	~~Hawkesbury, AUS~~	~~(33.570°S/ 150.77°E)~~	~~grass~~	~~+~~	~~-~~	~~-~~	~~5.4~~	~~0.03~~
~~*1000 ppt OCS 'elevated' || 400 ppm CO_2 ‚ambient'*~~								
~~A1~~				~~+~~	~~+~~	~~+~~		

~~* data adopted from Bunk et al., 2017, ** data adopted from Behrendt et al., 2014, n. d. not determined.~~

Soil ID	Location	Coordinates	Vegetation cover	CO/NO** [pmol g h^{-1}]	Inc. time [h]	NH_4 [mg kg^{-1}]	NO_3 [mg kg^{-1}]	pH	S [%]
500 ppt OCS 'ambient' & 400 ppm CO_2 ,ambient'									
D1	Bahariyya, Egypt	(28.362°N/ 28.860°E)	-	-	22	3.7	37.7	8.3	0.13
D2	Waxxari, China	(38.705°N/ 87.414°E)	-	-	25	<1.0	325.0	8.3	3.74
F1	Canarana, Brazil	(13.077°S/ 52.377°W)	rainforest natural	-	64.6	54.1	10.4	4.6	0.02
F2	Canarana, Brazil	(13.079°S/ 52.386°W)	rainforest burned	-	29	18.3	7.4	4.5	n.d.
A1	Mainz, Germany	(49.951°N/ 08.250°E)	corn	-	71	<0.05*	3.78*	7.6*	0.03*
A2	Baldingen, Germany	(48.865°N/ 10.462°E)	corn	-	71	<0.1*	86.0*	7.1*	0.03*
A3	Baldingen, Germany	(48.866°N/ 10.866°E)	sugarbeet	-	71	1.6*	75.6*	7.2*	0.04*
A4	Baldingen, Germany	(48.867°N/ 10.467°E)	wheat	-	50	1.9	29.0	7.7	0.03
A5	Hawkesbury, Australia	(33.570°S/ 150.77°E)	grass	-	38.3	2.9**	17.5**	5.4**	0.03

50 ppt OCS ,zero air' & 400 ppm CO_2 ,ambient'					
A1	Mainz, Germany	(49.951°N/ 08.250°E)	corn	+	96.6
A1dry	Mainz, Germany	(49.951°N/ 08.250°E)	corn	+	96.6
F3	Sparneck, Germany	(50.143°N/ 11.867°E)	spruce	+	

1000 ppt OCS 'elevated' & 400 ppm CO_2 ,ambient'					
A1	Mainz, Germany	(49.951°N/ 08.250°E)	corn	+	61.4
A1dry	Mainz, Germany	(49.951°N/ 08.250°E)	corn	+	61.3
F3	Sparneck, Germany	(50.143°N/ 11.867°E)	spruce	+	

Note that OCS fluxes for F3, A1 and A1dry -are presented in Bunk et al., submitted.

* data adopted from Bunk et al., 2017, **data adopted from Oswald et al., 2013, n. d. not determined.

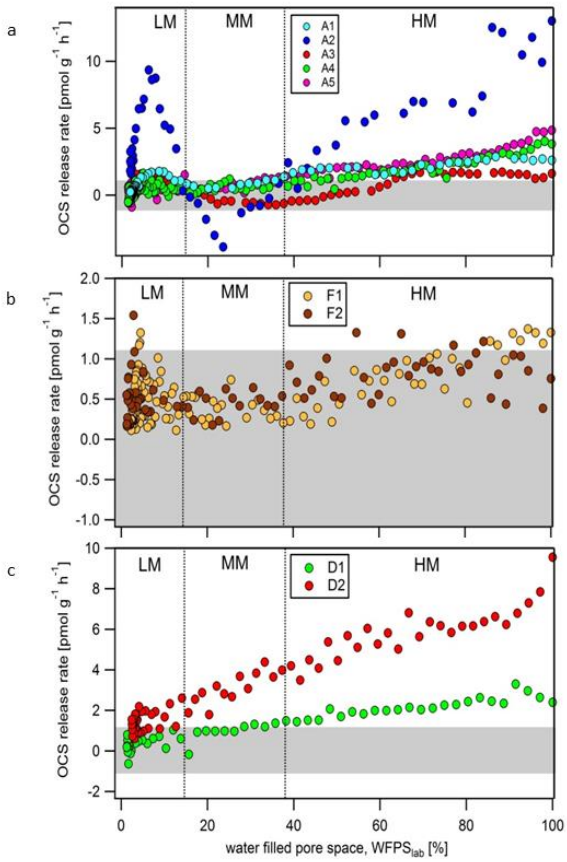

Fig. 1 OCS exchange rates from soil samples originated from agriculture (**a**) A1 to A5: cornfield (light blue), cornfield (dark blue), sugar beet (red dots), wheatfield (green), and grassland (pink), (**b**) F1, F2: natural rainforest (orange) and annual burned rainforest (brown), and (**c**) D1, D2: sand desert (green) sand desert (red) measured at 500 ppt OCS mixing ratio and 400 ppm CO_2 mixing ratio. Data of A1, A2, A3 are adapted from Bunk et al., submitted. Grey shaded area represents the threshold of 1.09 to -1.09 pmol g^{-1} h^{-1} where no significant OCS exchange could be detected.

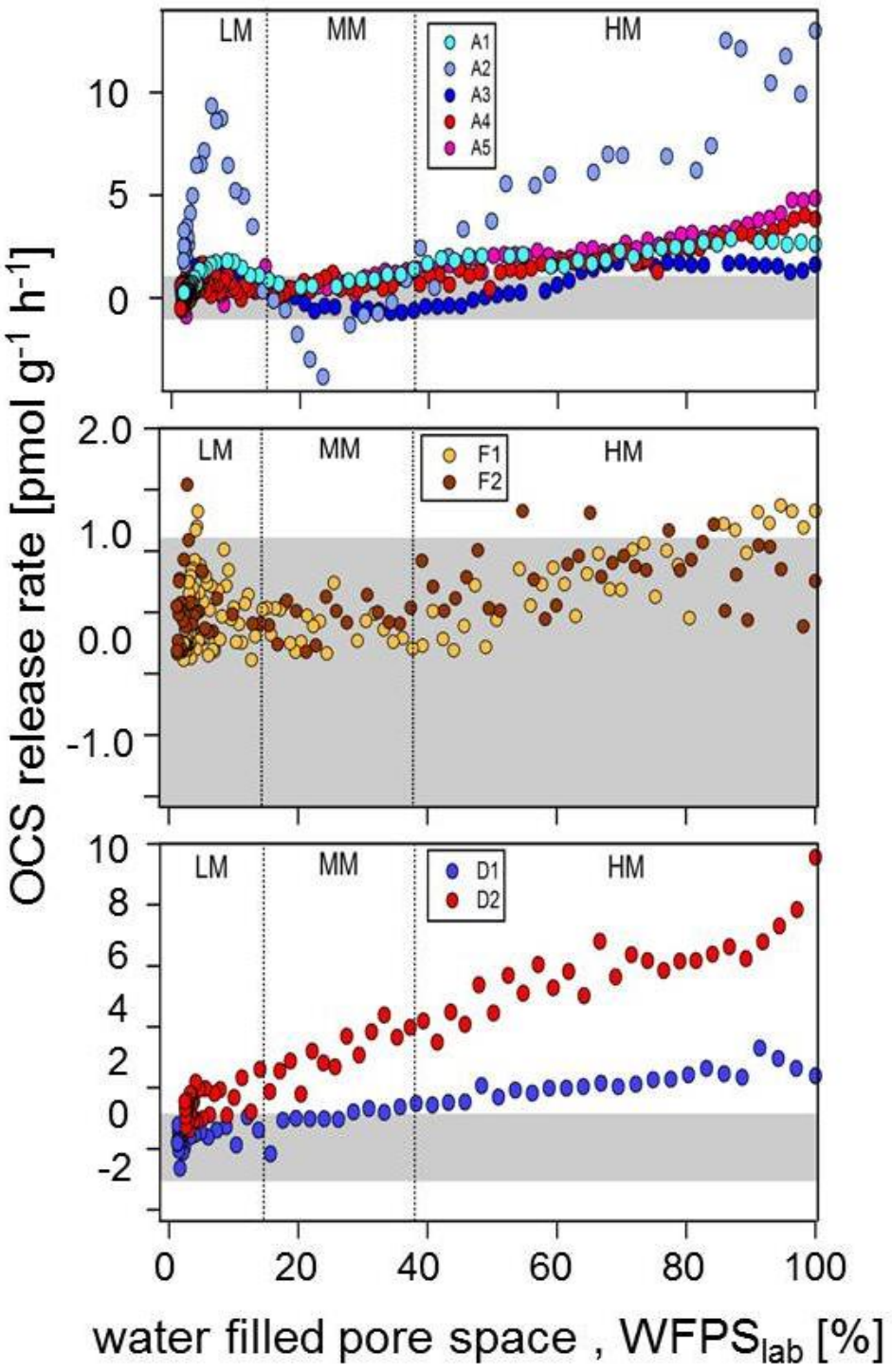

Figure 1 OCS exchange rates from soil samples originated from agriculture (**a**) A1 to A5: cornfield (light blue), cornfield (blue), sugar beet (dark blue), wheatfield (red), and grassland (pink), (**b**) F1, F2: natural rainforest (orange) and annual burned rainforest (brown), and (**c**) D1, D2: sand desert (blue) sand desert (red) measured at 500 ppt OCS mixing ratio and 400 ppm CO_2 mixing ratio. According to Bunk et al., 2017 OCS release rates are classified into high moisture (HM), moderate moisture (MM) and low moisture (LM) regime. Y-axix has different scales in subfigures. Data of A1, A2, A3 are adapted from Bunk et al., submitted. Grey shaded area represents the threshold of 1.09 to -1.09 pmol g^{-1} h^{-1} where no significant OCS exchange could be detected.

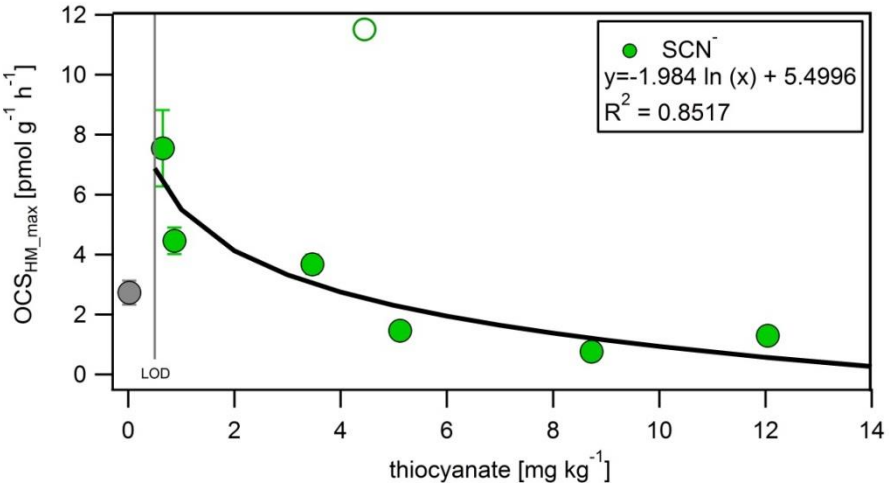

Fig. 2 Correlation between OCS exchange rate, $OCS_{max,HM}$ and thiocyanate (SCN^-) at high soil moisture for samples F1, F2, A3, A4, A5 (green). The maximum OCS exchange rate and thiocyanate concentration for A2 (green circle) are considered as an outlier, possibly due to release of thiocyanate from fine roots during the sieving procedure. Thiocyanate was below limit of detection (LOD of 0.5 mg kg^{-1}) for D1 soil (grey).

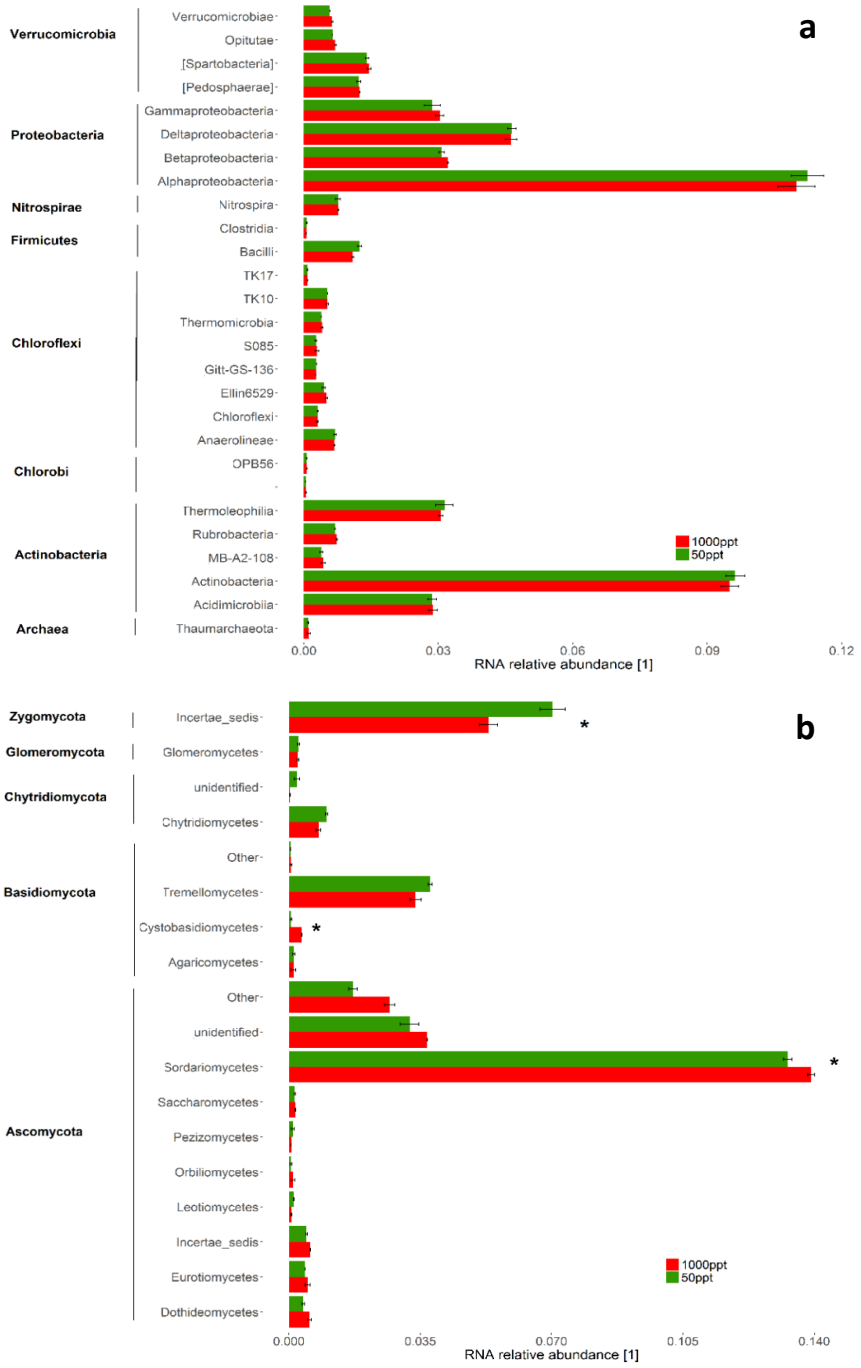

Fig. 3 Taxonomic composition of the mid-latitude corn field soil Mainz, Germany, at 22 % $WFPS_{lab}$ of the samples under 1000 ppt or 50 ppt OCS. Relative abundance of (a) 16S rRNA transcripts for selected bacterial classes and (b) internal transcribed spacer (ITS) transcripts for fungal classes, normalized by the total number of assigned reads per sample. Classes with RNA relative abundance < 5×10^{-4} did not show significant differences and were not plotted. Error bars represent standard deviation. Asterisks represent statistically different values (p-value < 0.05). Classes named as "unclassified" or "Other" are groups identified by the Qiime pipeline, however with no known classification in the database, under the used threshold of sequence similarity (90 %).

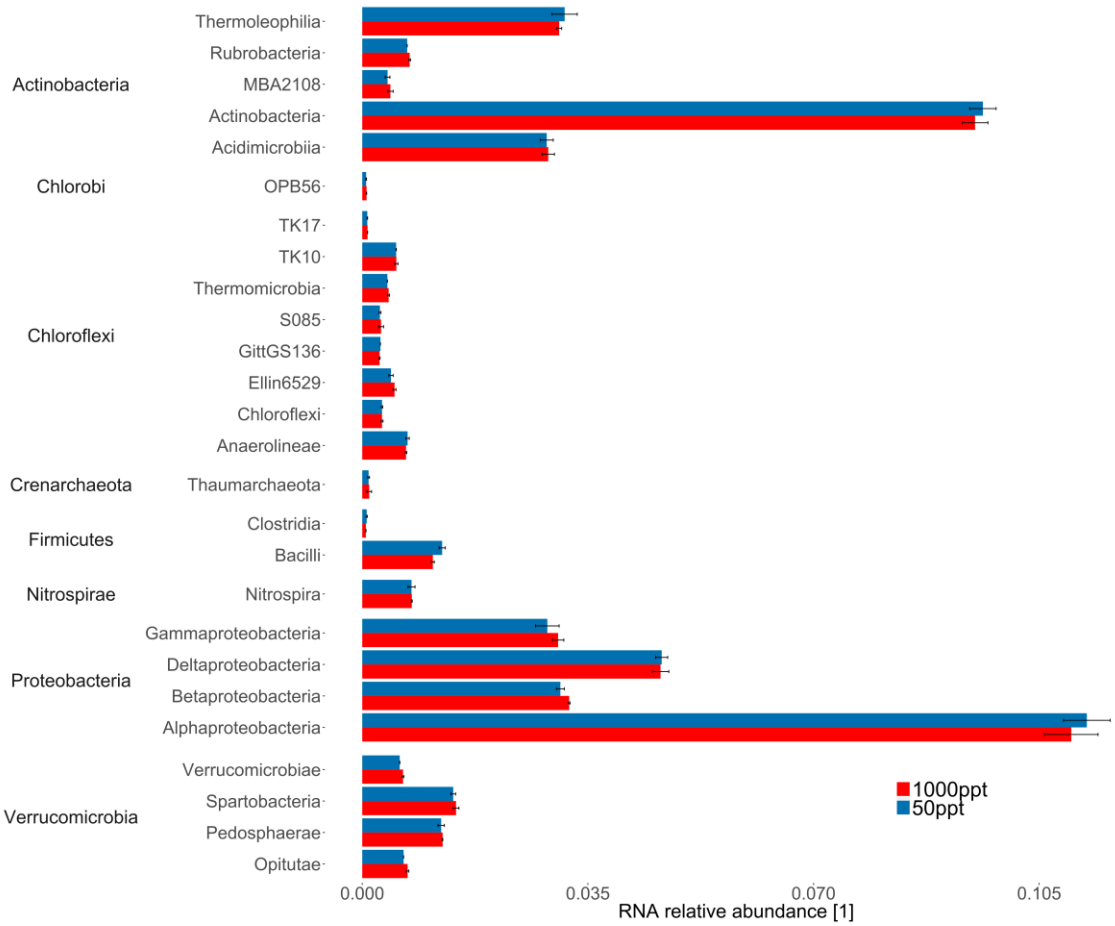

Figure 3 Taxonomic composition of the mid-latitude corn field soil Mainz, Germany, at 22% $WFPS_{lab}$ of the samples under 1000 ppt or 50 ppt OCS. 16S rRNA relative abundance for selected bacterial classes have been normalized by the total number of assigned reads per sample. Classes with RNA relative abundance < 5 x 10^{-4} did not show significant differences and were not plotted. Error bars represent standard deviation. Asterisks represent statistically different values (p-value < 0.05).

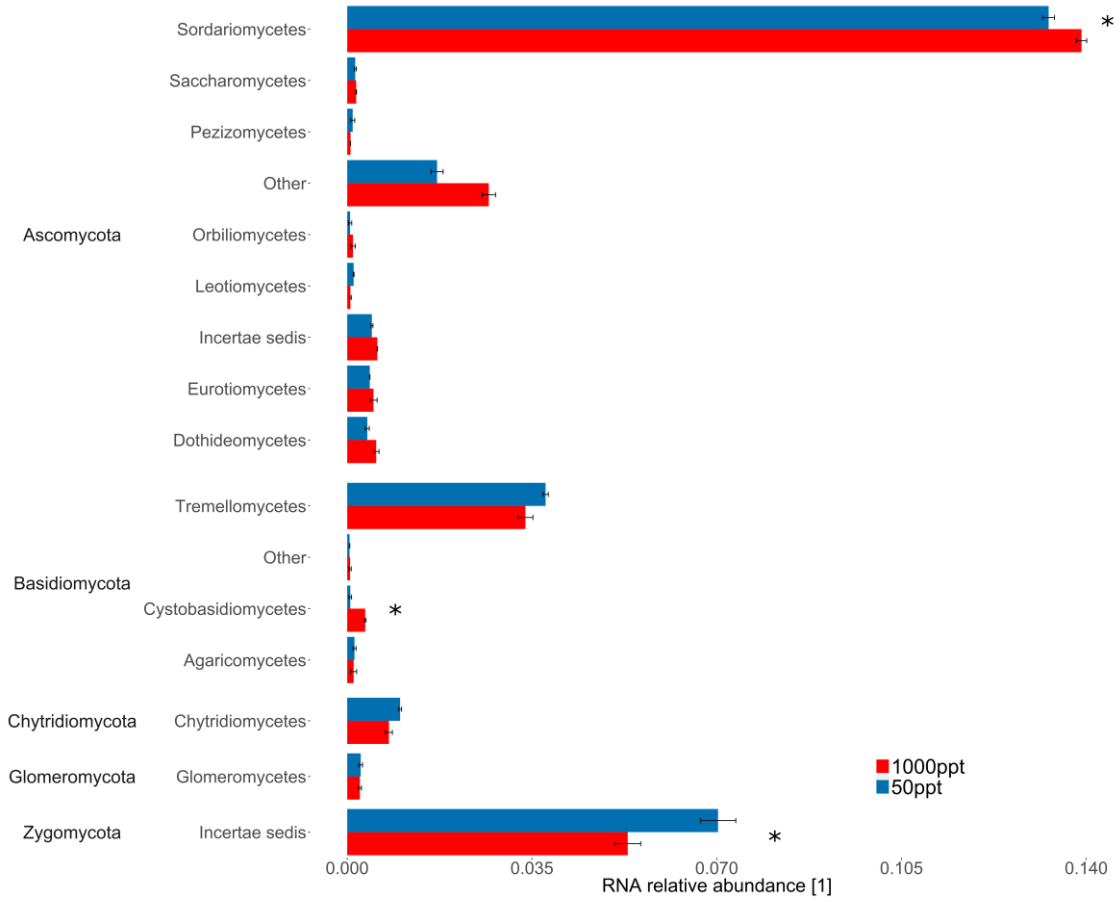

Figure 4 Taxonomic composition of the mid-latitude corn field soil Mainz, Germany, at 22% $WFPS_{lab}$ of the samples under 1000 ppt or 50 ppt OCS. RNA relative abundance of internal transcribed spacer (ITS) for fungal classes have been normalized by the total number of assigned reads per sample. Classes with RNA relative abundance < 5 x 10^{-4} did not show significant differences and were not plotted. Error bars represent standard deviation. Asterisks represent statistically different values (p-value < 0.05). "Other" is identified by the Qiime pipeline, however with no known classification in the database, under the used threshold of sequence similarity (90%).

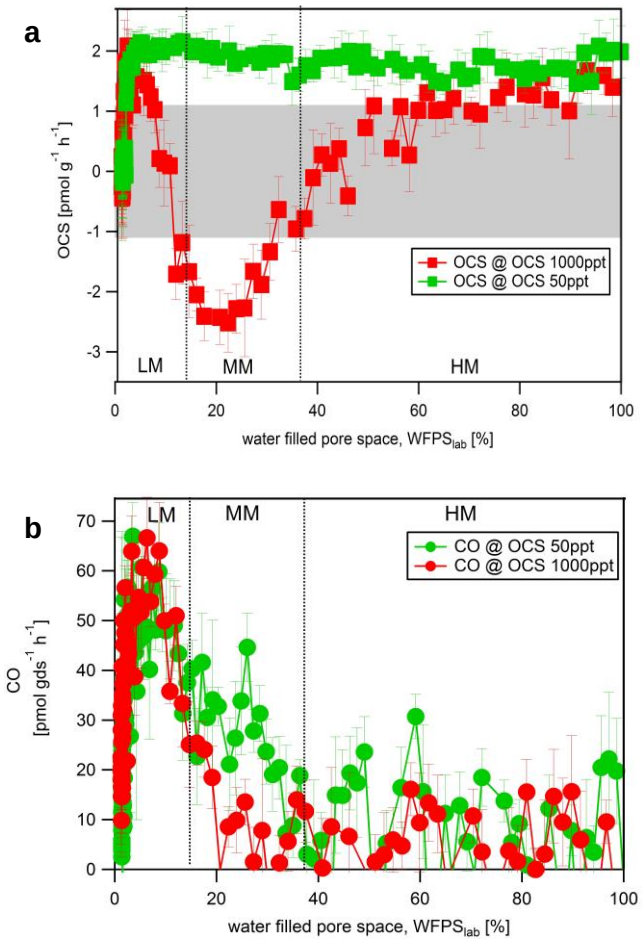

Fig. 4 OCS exchange rates (a) and CO exchange rates (b) at OCS mixing ratio of 50 ppt (green) and 1000 ppt (red) are shown for the A1 soil sample from a mid-latitude corn field, Mainz, Germany, data for (a) adapted from Bunk et al., submitted. Grey shaded area represents threshold 1.09 to -1.09 pmol g^{-1} h^{-1} where no significant OCS exchange could be detected. LM, MM and HM indicate low, medium and high moisture levels, respectively.

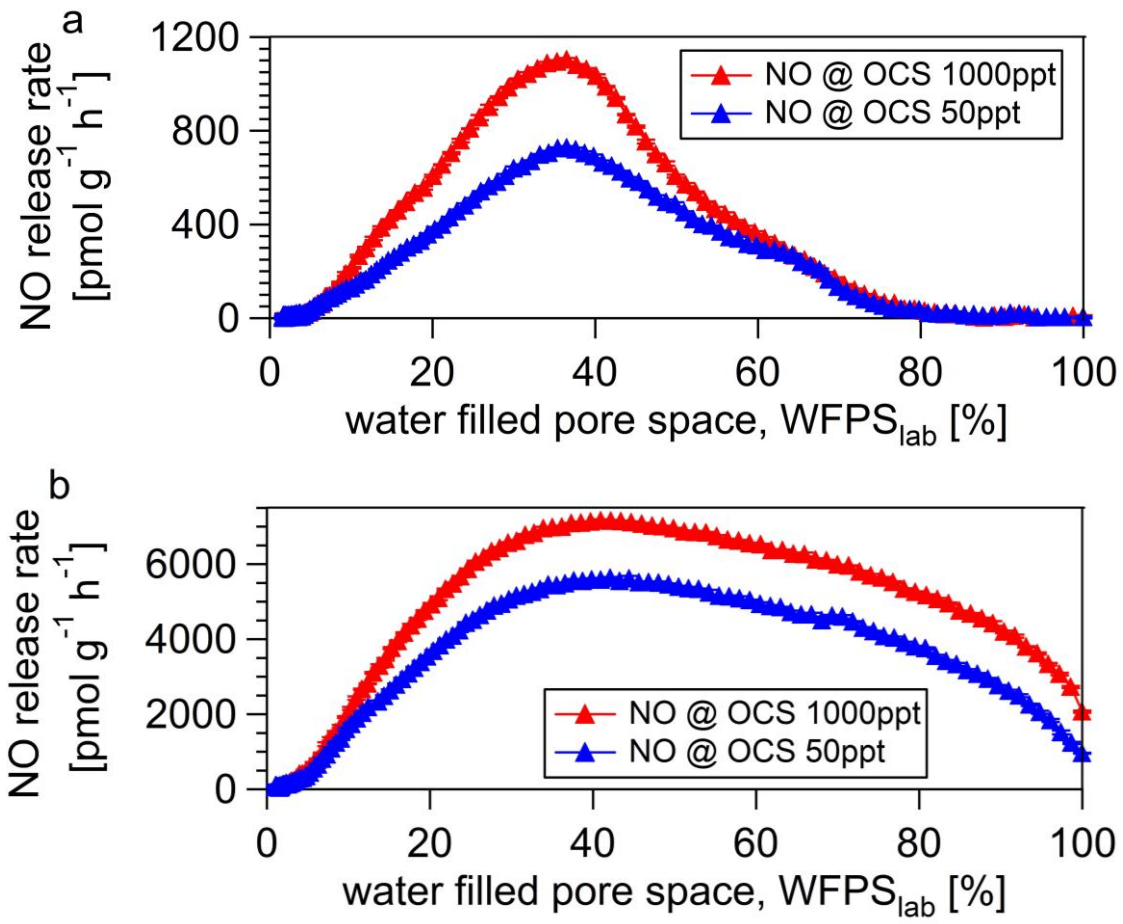

Figure 5 NO exchange rates (a) are shown for a mid-latitude cornfield soil sample 40°C dried from Mainz, Germany (A1) and a soil sample originated from a spruce forest Sparneck, Germany at OCS mixing ratio of 50 ppt (blue) and 1000 ppt (red).

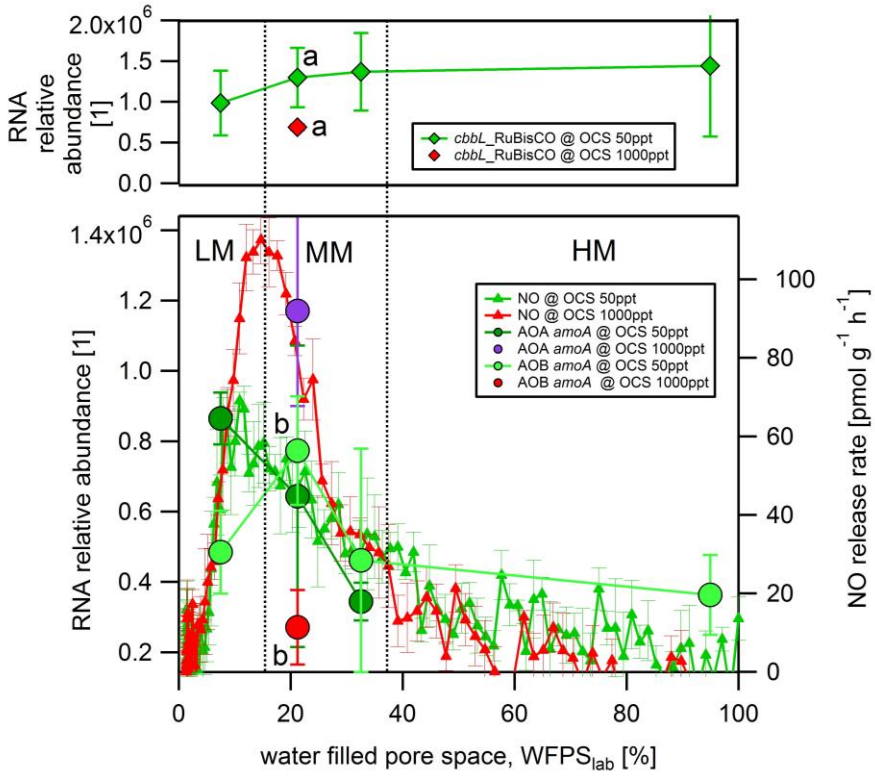

Fig. 5 RNA relative abundance of *cbbL* functional gene, encoding Ribulose-1,5-Bisphosphate-Carboxylase (RuBisCO) large subunit type IA, measured over dry-out under 50 ppt OCS (green diamonds) and 1000 ppt OCS (red diamond). RNA relative abundance of *amoA* functional gene for ammonia oxidizing bacteria (AOB, bright green points) and ammonia oxidizing archaea (AOA, dark green points) measured over dry-out under 50 ppt OCS and 1000 ppt OCS (AOB, orange point and AOA light green point). NO exchange rates at 50 ppt (dark blue) and 1000 ppt (light blue) OCS mixing ratio are shown for the A1 soil sample from a mid-latitude corn field, Mainz, Germany. Note values for *amoA* AOB are multiplied by 100 and differences in RNA relative abundance under 50 ppt and 1000 ppt are statistically significant (p-value < 0.05, a, b).

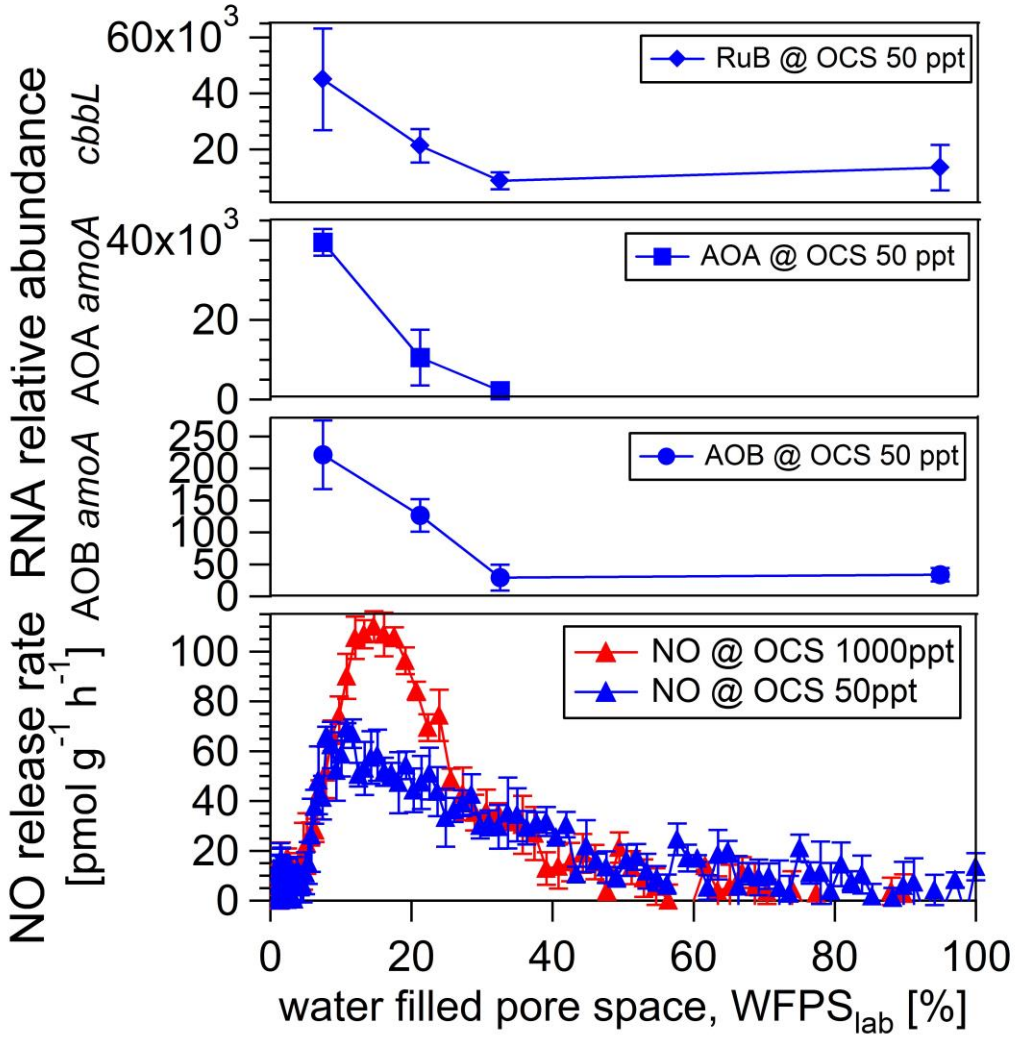

Figure 6 RNA relative abundance of *cbbL* functional gene, encoding Ribulose-1,5-Bisphosphate-Carboxylase (RubisCO) large subunit type IA, measured over dry-out under 50 ppt OCS (blue diamonds). RNA relative abundance of *amoA* functional gene for ammonia oxidizing archaea (AOA, blue squares) and ammonia oxidizing bacteria (AOB, blue points) measured over dry-out under 50 ppt OCS. NO exchange rates at 50 ppt (dark blue) and 1000 ppt (light blue) OCS mixing ratio are shown for the A1 soil sample from a mid-latitude corn field, Mainz, Germany.

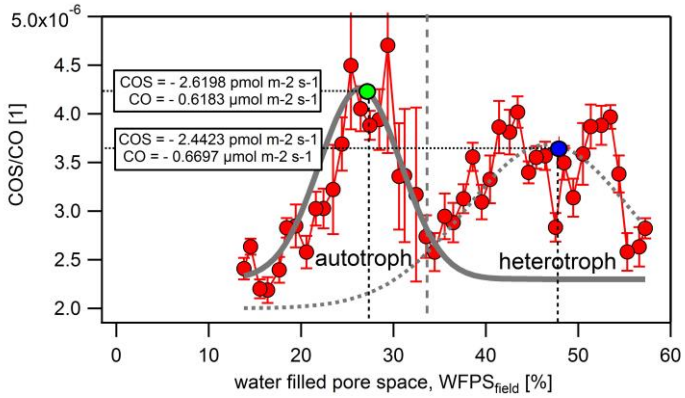

Fig. 6 OCS:CO ratio reanalyzed from chamber measurements from Sun et al. (2017) field data in a Scots pine forest from Hyytiälä normalized by assuming Q_{10} value equals 2. Just as denitrification and nitrification affect N_2O:NO ratios differently, we assume 2 different processes (one autotrophic and one heterotrophic) were simultaneously involved in OCS exchange and CO consumption, one dominating under elevated and the other under moderate soil moisture (indicated as grey optimum functions).

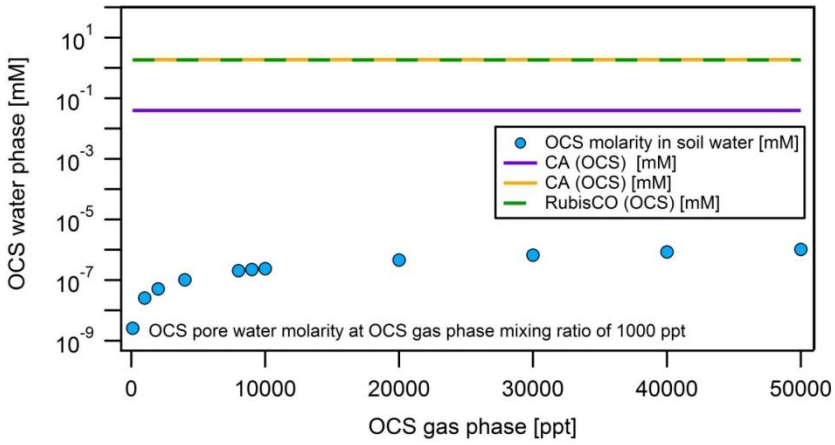

Fig. 7 The range of K_M values of carbonic anhydrase (purple and orange) and RubisCO (green) compared to the calculated OCS concentration in the water phase (blue). The expected water phase concentration was calculated in a similar approach than in Bunk et al. (2017) from the known gas phase concentration following Henry's law. The K_M values are medians of data reported in the BRENDA database (see section 4.4).